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ROYAL COMMISSION ON TUBERCULOSIS (HUMAN AND BOVINE).

FINAL REPORT OF THE ROYAL COMMISSION

APPOINTED TO INQUIRE INTO THE RELATIONS OF

HUMAN AND ANIMAL TUBERCULOSIS.

PART II. APPENDIX

VOLUME V.

Comparative Histological and Bacteriological Investigations.

ARTHUR EASTWOOD, M.D.

Presented to both Houses of Parliament by Command of His Majesty.

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PREFACE.

THIS Report is the continuation of my previous Report on "The Comparative Histological and Bacteriological Investigations." (App. to 2nd Int. Rep. Vol. IV.)

Additional details are here given of the changes produced by bovine and by human viruses in the tissues of experimental animals. To these are added descriptions of animal tissues infected with mammalian tubercle bacilli isolated from swine, horses, a gnu, and an antelope. The histology of various species of animals experimentally infected with avian tubercle bacilli is also dealt with.

The bacteriological part of the present Report, following the same order, contains descriptions of the cultural characters of the various examples of tubercle bacilli which have been investigated.

A comparison is made of the various mammalian viruses investigated, and also of the mammalian as contrasted with the avian viruses.

Preceding the histological and bacteriological details, there is a general account of the results of my work on the comparative histology and bacteriology of tuberculosis.

For the tissues submitted to histological examination and for the original cultures, the characters of which were subsequently investigated by me, I am indebted to my colleagues Drs. A. S. and F. GRIFFITH.

For the original drawings and the lithographic reproductions I am indebted to the skill of Mr. J. R. FORD.

ARTHUR EASTWOOD.

Royalcot Laboratory,
Stansted,
Essex.

December, 1909.

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PART I.

RESULTS OF THE INVESTIGATIONS.

THE HISTOLOGICAL RESULTS WITH MAMMALIAN VIRUSES.

ADDITIONAL OBSERVATIONS ON LESIONS PRODUCED BY BOVINE VIRUSES.

The work with bovine viruses was almost completed when my last Report was written. Since then no new bovine viruses have been received for the purpose of comparative investigation; but my colleagues, Drs. A. S. and F. Griffith, have made some additional experiments with the viruses already in hand, and these have afforded me material for further histological observations.

(1) *In Bovines.*

In my last Report I showed that when subcutaneously inoculated into young calves in a dose of 50 mg. of culture, the viruses of bovine origin produced lesions which were histologically typical of highly acute tuberculosis. Since then I have had the opportunity of examining the tissues of calves which had received a similar dose after treatment, some time previously, with other inoculations of living tubercle bacilli. My observations on the tissues of these animals, with reference to the histological evidence of susceptibility or resistance, may be summarised as follows:—

(1) The subcutaneous inoculation, 51 days previously, of ·001 mg. fo B XXVI greatly reduced the susceptibility of the tissues of two calves, each five weeks old, to a dose of 50 mg. of the same virus.

(2) An equally marked reduction in susceptibility to B XXVI in like dose was produced in two calves, one five and the other eight weeks old, by the previous inoculation subcutaneously of 50 mg. of a human virus possessing low virulence for the bovine.

(3) When the same slightly virulent human virus was introduced intravenously, in a dose of 10 mg., into two calves, each six weeks old, the results as shown by histological examination were unequal. One animal proved highly resistant to the subsequent test inoculation with virulent bovine bacilli; the other succumbed to fatal infection.

(4) Previous treatment with two doses of slightly virulent human viruses (50 mg. subcutaneously followed by 50 mg. intravenously) rendered the tissues of two calves, eight and twelve weeks old, much less susceptible to subcutaneous inoculation with 50 mg. of B XXVI; but the increased resistance was not more marked than in the case of two younger calves (five and eight weeks old), which received only one dose of slightly virulent bacilli (50 mg. subcutaneously).

(5) Previous treatment with 10 mg. of slightly virulent human bacilli (intravenously), followed by a second vaccination with 1 mg. of B XXVI (subcutaneously), increased the resistance of two calves (nine and eleven weeks old) to 50 mg. of B XXVI subcutaneously inoculated; but the increased resistance was not more marked than in the case of two younger calves (each five weeks old) which received only one vaccination (·001 mg. of B XXVI subcutaneously).

(6) Previous treatment with two protective subcutaneous doses (10 mg. followed by 50 mg.) of slightly virulent human bacilli produced unequal results in two calves, each aged thirteen weeks. One showed very high resistance; the other was fatally infected.

The above histological results, though far from uniform, may, it appears to me, at least afford ground for presumption that previous inoculation, either with a minute dose of highly virulent bacilli or with a relatively large dose of less virulent bacilli, tends to increase the animal's powers of resistance. The increase of age in the period between the first protective inoculation and inoculation with the test dose must also be taken into account, since with older animals, which have not been thus vaccinated, it is not certain, as is apparent from the tissues which I have investigated, that 50 mg. of bovine bacilli will produce rapidly fatal disease. A dose of 50 mg. of B XXVI, inoculated subcutaneously into two such normal calves, produced unequal results, the infection being mild in one animal (seventeen weeks old) and rapidly fatal in the other (nineteen weeks old); these experimental results were confirmed by my histological investigations.

(2) *In Other Mammals.*

The rabbit, as I have already shown by histological examination, is highly susceptible to the bovine bacillus, but it appears that after subcutaneous inoculation of this animal disseminated lesions are relatively slow in making their appearance. In rabbits which have received a subcutaneous dose of 50 mg. of bovine bacilli I have found fewer disseminated tubercles during the first fortnight than in calves, after the same dose, or in swine, after a dose of 20 mg.

Even when previously killed by heating, bovine bacilli exert an irritant and toxic effect upon the tissues of the rabbit, and large deposits of such bacilli produce typical tubercles of a chronic type.

It has already been established that anthropoids and monkeys are highly susceptible to the bovine bacillus, whether administered by feeding or by inoculation. Additional examples of the effects of a very minute dose of bovine bacilli subcutaneously inoculated are provided by Baboon 76 and Chimpanzee 24. In both cases there was general dissemination of the injected bacilli, and in both animals the lungs were more extensively affected than other organs. The chimpanzee showed histological lesions of a more acute type than those of the the baboon ; this is in accordance with my previous results, which show that the chimpanzee is of even greater susceptibility than the baboon.

In Pig 138 the subcutaneous inoculation of 1 mg. of bovine bacilli produced disseminated lesions which, I found, had become definitely retrogressive at the end of three months and which contained no demonstrable bacilli in the specimens I examined. This example, like others previously recorded, illustrates the early dissemination of tubercle bacilli in the pig associated with the development of the lesions thus formed into tubercles of a chronic and retrogressive type.

Pig 216 exhibited disseminated tuberculosis of a chronic type after ingesting faecal material obtained from a cow suffering from spontaneous tuberculosis.

Ponies 6 and 12 appear to have exhibited complete resistance to the subcutaneous inoculation of 50 mg. of bovine bacilli. But in Pony 30 the intravenous inoculation of 10 mg. produced histological evidence of fatal tuberculous infection of the lungs.

(3) *In Fowls and Parrots.*

The fowl, judging from the specimens I have examined, does not appear to be susceptible to the bovine bacillus except by the intravenous method of experiment. Intravenous inoculation has sometimes produced in the lungs of fowls caseous tubercles of a chronic type. In these lesions I have found that the bacilli may survive for a long period (e.g., 100 days) and may show some evidence of multiplication. In the other organs of the fowl bovine bacilli thus introduced do not seem capable of lengthy survival. It is noteworthy that in Fowl 492, intravenously inoculated with dead bacilli, there is as much tissue change as is usually found in fowls inoculated with an equal amount of living mammalian bacilli.

Parrots are more susceptible than fowls to the bovine bacillus, extensive disease being produced in them by intramuscular inoculation and by feeding, as well as by intravenous inoculation. The bacilli often multiply freely, particularly in their lungs, which, as in fowls, appear to be the most susceptible tissue.

ADDITIONAL OBSERVATIONS ON LESIONS PRODUCED BY HUMAN VIRUSES.

Since the publication of my last Report the work on human viruses has been in progress continuously and has provided me with a large amount of additional material for histological investigation. This material has been supplied to me by my colleague Dr. A. S. Griffith.

(1) *In Bovines.*

In a large number of calves fatal disease has been produced by the subcutaneous inoculation of culture of bacilli derived from human sources, the dose being generally 50 mg. In histological details the lesions produced in these fatally infected animals, more especially in their lungs, are very much alike and are typical of highly acute infection. In some instances evidence of less severe infection than that of the lungs has been noted in one or other organ ; but these exceptions are relatively slight, and in all the calves in question the histological evidence is sufficient to show that the bacilli inoculated had produced fatal disease. The tissues examined were from experiments which terminated at periods varying from 19 to 73 days after inoculation and include Calves 1567, 1579, and 1585, which were inoculated with the sputum viruses H 127. R.R. and H 128. D.D.

Examinations have also been made of numerous calves in which less severe disease was produced by subcutaneous inoculation of bacilli derived from human sources. As I pointed out in my last Report, when a large dose, such as 50 mg. of culture of such virus, is inoculated subcutaneously, the main issue of the experiment is decided in the subcutaneous tissue at the site of inoculation. This tissue is commonly incapable of offering a successful resistance to a large dose of highly virulent bacilli, and hence their introduction is generally followed by rapidly fatal dissemination. But when a like dose of less virulent bacilli is introduced, the local tissue reaction is nearly always sufficiently effective to prevent the dissemination of the greater part of the inoculated material. The bulk of the bacilli become surrounded at the site of inoculation by a dense, leathery zone of fibrous tissue which completely excludes most of them from access to the rest of the body. Hence the results of subcutaneous inoculations tend to fall into one of two widely distinct classes, characterised by acute dissemination in the one case and, in the other, by the limitation of disseminated disease, when such is present, to isolated tubercles usually of retrogressive type. Many of the calves dealt with in the present Report come under this latter class. In some cases these isolated tubercles are few in number, small in size, and very definitely retrogressive. In other instances they are numerous and form fairly large caseous foci which, though apparently quiescent, are less definitely in a retrogressive stage. And in a few examples the disease is still more extensive and may be described as intermediate in severity between the acute and the chronic. Calf 1237, inoculated with a tissue emulsion containing bacilli belonging to the virus H 13. A.D., and Calf 1213, inoculated with 50 mg. of the virus H 49. T.C., afford examples of this intermediate condition.

Attention may be called to the anomalies of the results obtained with certain viruses.

H 49. T.C.—Two calves (1213 and 1501) were inoculated each with 50 mg. of culture (derived from original material) ; in the tissues of the latter the disease was definitely acute ; in the former it was less acute and may be described as being of intermediate severity. A tissue emulsion from Calf 1213 produced only a mild type of disease in Calf 1263 ; but 50 mg. of culture from this Calf 1263 produced acute disease in Calf 1347. The dose of bacilli contained in a tissue emulsion is much less than in 50 mg. of culture, and it is known that tissue emulsions containing highly virulent bacilli do not necessarily produce severe disease in calves ; the mildness of the infection in Calf 1263 has therefore no special significance. Omitting this case, the amount of discrepancy in my other results is only slight, and may be summed up by saying that, in so far as my histological observations extend, the virulence of H 49. T.C. is high, but less uniformly high than that of a typical bovine virus.

H 53. D.H.—Material from the patient yielding this virus was obtained on two separate occasions. The material first obtained (a) yielded bacilli possessing relatively low virulence for the bovine (Calves 1135, 1435, and 1477). The second material (b), obtained three years and eight months later, proved to be of high bovine virulence in two instances which I examined (Calves 1507 and 1545), and of low bovine virulence in another (Calf 1535).

H 60. W.B.—The bacilli from the bronchial gland of the human subject yielding this virus produced, in the bovine, lesions showing high virulence (Calves 1099, 1107, and 1109) ; but bacilli obtained at the same time from other tissues of the same case (meninges and lungs) were of low virulence for the bovine animal.

H 69. F.K.—Bacilli derived from the human meninges of this case produced acute disease (Calf 1267) ; but the bacilli obtained at the same time from the mesenteric gland produced only mild infection (Calf 1249).

H 89. W.I.—This virus produced severe disease in one animal (Calf 1351) and mild disease in another (Calf 1275).

H 90. I.P.—Two calves (1383 and 1471) inoculated with bacilli obtained by culture from a retro-peritoneal gland of the human subject showed only slight disease, but a culture from the former calf produced severe disease in Calf 1489, and a culture from Calf 1471 produced severe disease in Calf 1493.

*H 100. R.S.**—In Calves 1409 and 1419 lesions of a chronic type were produced by cultures from the original material ; but in Calf 1523 culture from the same source produced acute infection. A culture from Calf 1409 produced acute infection in Calf 1543 ; and a culture from Calf 1419 produced the same result in Calf 1517.

*H. 105. G.S.**—Cultures from original material produced lesions of a chronic type in Calves 1405 and 1449 ; but a culture from Calf 1405 produced acute infection in Calf 1527, and a culture from Calf 1449 produced the same result in Calf 1521.

*H 107. H.H.**—Cultures from original material produced mild infection in Calves 1453 and 1497. A culture from Calf 1497 produced disseminated lesions of chronic type in Calf 1549. A culture from Calf 1453 produced only slight infection in Calves 1505 and 1513 but typically acute disease in Calf 1519.

*H 108. H.R.**—Cultures from original material produced only slight infection in Calves 1417 and 1421. But a culture from Calf 1417 produced typically acute infection in Calf 1539, and a culture from Calf 1539 produced a similar result in Calf 1509.

(2) *In Monkeys.*

Usually, but not invariably, histological examination has shown that the rhesus monkey is highly susceptible, even to the less virulent varieties of human tubercle bacilli. The following are examples of results, obtained with human bacilli exhibiting lower virulence than bovine bacilli, which present some deviation from this general rule.

H 8. S.C.—The three monkeys inoculated with ·01, ·1, and 1 mg. of this virus showed a relatively mild type of disease.

H 53. D.H. (*a*).—Three out of five animals inoculated with from ·1 to 10 mg. of this virus showed remarkably little disease ; in the two others there was more tissue damage but the infection was not particularly acute, the doses in these last cases being 10 mg. and ·1 mg.

H 60. W.B. (*Mesenteric Gland*).—Feeding with 1 mg. of culture produced relatively mild disease in Monkey 81 but acute disease in Monkey 83.

H 85. H.B.—Disseminated disease of a mild type was produced in Monkey 125 after feeding with 1 mg. of culture. In Monkey 119 severe disseminated tuberculosis was caused by the subcutaneous inoculation of ·01 mg. In Monkeys 121, 123, and 153 subcutaneous inoculation was followed by less severe disease, the doses being, respectively, ·1 mg., 1 mg., and 10 mg.

H 91. H.S.—In Monkey 133 only slight dissemination was produced by the subcutaneous inoculation of ·1 mg.

H 100. R.S. (*Original material*).—In Monkey 163, after subcutaneous inoculation with 1 mg. of culture, the disease was not particularly acute. There were abundant caseous foci in the lungs and spleen, but bacilli were very scanty.

H 102. N.H.—In Monkey 213 the subcutaneous dose of 1 mg. produced disseminated tuberculosis of a chronic type.

H 109. M.W.—A subcutaneous dose of ·1 mg. produced relatively slight disease in Monkey 185 ; but in Monkey 197 the larger subcutaneous dose of 1 mg. produced acute infection.

H 114. A.U.—In Monkey 223 a dose of 1 mg., subcutaneously, produced disseminated and moderately acute tuberculosis. In Monkey 225 a like dose produced less severe disease.

As the above mentioned viruses, with the exceptions of H 8. S.C. and H 60. W.B., were derived from human lupus, it appears to me possible that residence in the human skin may have caused some attenuation of the bacilli.

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* These tests were all made by subcutaneous inoculation. In all cases where acute disease was produced the dose was 50 mg. ; in the other cases the dose varied from 50 mg. to 100 mg., with the exception of Calf 1449, where the dose was 48 mg.

(3) *In other Mammals.*

Additional histological examinations have been made of rabbits inoculated with human viruses which, while differing among themselves in degree of virulence, have all, except one, failed to exhibit the uniformly high virulence characteristic of the typical bovine virus. The results again show that the processes of tubercle formation are of the same essential nature but differ merely in respect of severity. Thus, H 77. R.L., which is equal in virulence to a bovine virus, in an intravenous dose of ·1 mg. of culture produced death in 23 days with typically acute disease and abundant multiplication of bacilli. With the same intravenous dose, H 49. T.C. and H 85. H.B. produced death in 31 and 48 days, respectively; the infections may be described as acute, but there was rather less tissue destruction and rather less abundant multiplication of bacilli than in very acute cases. With the larger intravenous dose of 1 mg., H 78. O.D. produced death in 18 days in one rabbit and death in 28 days in another: there was extensive disease, particularly in the lungs of the two animals, but the multiplication of bacilli was distinctly less than with more virulent viruses. In Rabbit 1205, after a like intravenous dose of H 75. A.H., the duration of life was much longer; the animal was killed 101 days after inoculation and then showed extensive disease of the lungs of a slowly progressive type. A series of rabbits, inoculated intravenously, each with 1 mg. of H 90. I.P., and killed at periods varying from 24 hours to 14 days after inoculation, also illustrates a much milder type of infection than that found in two corresponding series of rabbits inoculated with highly virulent bacilli. In a series of rabbits inoculated subcutaneously, each with 50 mg. of the last-mentioned virus (H 90. I.P.), and killed at periods ranging from 24 hours to 14 days after inoculation, no microscopic evidence of disseminated lesions has been found. In this connection it may be recalled that in a similar series inoculated with bovine bacilli the evidence of dissemination was only slight.

My histological observations on various swine, fed or subcutaneously inoculated with human bacilli possessing relatively low virulence for the bovine, show that no more than a mild and retrogressive type of disease is generally produced. But more severe disease has been caused in Pig 121 by the subcutaneous inoculation of 50 mg. of culture of H 53. D.H. The feeding experiments, as well as the subcutaneous inoculations, have, I find, often produced tubercles in the lungs.

In guinea-pigs inoculated intraperitoneally or subcutaneously with various human viruses possessing low virulence for the bovine there is evidence, in every case examined, of generalised and fatal tuberculosis. But in a guinea-pig which died 23 days after intraperitoneal inoculation of 100 mg. of dead bacilli practically no evidence of dissemination was found by me.

In the tissues of three dogs inoculated with human viruses of relatively low virulence for the bovine, two intraperitoneally and the third intravenously, the infection produced was quite as severe, histologically, as that caused in dogs by bovine viruses. There was general dissemination and abundant multiplication of bacilli, but an absence of tubercle formation.

In a horse a 50 mg. dose, subcutaneously, of a human virus of relatively low virulence for the bovine or rabbit, failed to produced evidence of infection in any of the tissues examined. A similar result was obtained with a pony inoculated subcutaneously with 50 mg. of a bovine virus. Horse 11, inoculated intravenously with 10 mg. of culture of H 85. H.B., died in 51 days, with general dissemination and abundant multiplication of bacilli but little tissue destruction. Horse 7, inoculated intravenously with a like dose of H 92. D.N., showed very high resistance to infection.

Amongst the additional specimens of goats examined it may be noted that Goat 69, inoculated subcutaneously with 10 mg. of a human virus of relatively low virulence, exhibited extensive chronic tuberculosis of the mammary gland, and that Goat 81, inoculated intravenously with 2 mg. of a human virus also of relatively low virulence, showed generalised dissemination of bacilli without typical tubercle formation.

(4) *In Fowls and Parrots.*

In the specimens examined it has been found that human bacilli possessing relatively low virulence for the bovine thrive less readily and produce less tissue damage in these animals than bovine bacilli.

LESIONS PRODUCED BY MAMMALIAN TUBERCLE BACILLI ISOLATED FROM SWINE.

(1) *In Bovines.*

With one exception, the seven porcine viruses that I examined of those which had been tested upon calves have proved almost identical, in their histological effects, with viruses of bovine origin ; but perhaps P XIV, though of bovine type, is slightly below the average in respect of virulence.

Of the definite exceptions, the virus P IV, in subcutaneous doses of 50 mg., produced lesions histologically characteristic of slowly progressive tuberculosis in Calf 312, but no microscopic evidence of dissemination was afforded in the other calves similarly tested, with the exception of one, which showed small, retrogressive tubercles ; it was not in good health at the time of inoculation and died of intercurrent disease. The other two slightly virulent viruses, P XLI and P. XLVI, I am informed by Dr. F. Griffith, failed to produce disseminated lesions in calves beyond the glands nearest the site of inoculation.

(2) *In other Animals.*

The experiments on rabbits, swine, guinea-pigs, and other animals reveal no new features of histological interest in the specimens which I have examined.

LESIONS PRODUCED BY EQUINE TUBERCLE BACILLI.

(1) *In Horses.*

On intravenous inoculation in doses of 10 mg. the viruses E I, E II, and E IV have produced only slight tissue changes, and have not led to the formation of tubercles. In most of the organs examined, bacilli were present, but they did not, as a rule, show signs of multiplication. Subcutaneous inoculation, in a dose of 50 mg., produced only slight disease in Pony 20, inoculated with E II, but E IV in similar dose caused general dissemination, with lesions intermediate between the acute and chronic types, in Pony 22.

(2) *In Bovines.*

E I.—The lesions produced in the bovine animal by subcutaneous inoculation with this virus are typical of highly acute infection and are identical in every respect with the effects produced by highly virulent viruses obtained from bovines, human beings, and swine.

E II.—I have examined the tissues of eleven calves subcutaneously inoculated with culture. The results are irregular, and may be summarised as follows :—

(a) *Cultures derived from Original Material.*—Calf 406 received a dose of 10 mg. ; the lesions in the lungs, though not acute in type, contained numerous bacilli and cannot be regarded as retrogressive. But in the companion animal, Calf 404, which received a like dose, only very slight evidence of disease was found. And in Calf 506, which received a dose of 50 mg., there was only slight evidence of dissemination.

(b) *Cultures from Calves inoculated as above from Original Material.*—(1) 50 mg. of culture from Calf 406 were inoculated into Calf 596 and produced slowly progressive tuberculosis of the lungs, but very little evidence of disease in the other organs examined. (2) Doses of 50 mg. of culture from Calf 504 (in which 50 mg. of culture from original material produced only slight disease) were inoculated into Calves 578, 580, and 626. In Calf 578 mild, retrogressive infection was produced. In Calf 580, which died in 29 days, the lesions were typical of highly acute infection. Calf 626 died in 54 days with progressive, disseminated tuberculosis, the lungs being typical of acute infection. (3) Doses of 50 mg. of culture from Calf 506 were inoculated into Calves 592, 594, and 628. In Calf 592 there was only slight evidence of dissemination. Calf 594 died in 16 days and showed general dissemination, the lesions in the lungs being acute in type. Calf 628, killed 90 days after inoculation, exhibited moderately severe infection of the lungs and spleen.

(c) *A culture obtained after two passages in Calves.*—A dose of 50 mg. of culture from Calf 596 (inoculated from culture of Calf 406) was inoculated into Calf 638. The animal was killed 40 days afterwards and showed general dissemination, the lesions in the lungs being typical of acute infection.

E III.—Calves 440 and 508 showed typically acute infection of the lungs after subcutaneous inoculation with 50 mg. of culture.

E IV.—I have examined the tissues of nine calves subcutaneously inoculated with culture from this source. The results are irregular, and may be summarised as follows :—

(a) *Cultures derived from Original Material.*—In Calf 410, inoculated with 10 mg., there was only very slight evidence of dissemination. In Calf 502, inoculated with 50 mg., no evidence of tuberculosis was found in the specimens examined. In Calf 620, also inoculated with 50 mg., a few small lesions in the spleen were the only evidence of disease found. But in Calf 498, which died 89 days after the subcutaneous inoculation of 50 mg., there was acute infection of the lungs, with very large numbers of bacilli, suggestive of intravenous inoculation, but very little dissemination elsewhere.

(b) *Cultures from Calves inoculated as above from Original Material.*—(1) 50 mg. of culture from Calf 408 produced in Calf 582 disseminated tubercles which appeared to be progressive in the spleen, but retrogressive in the other tissues examined. (2) 50 mg. of culture from Calf 498 failed to produce any evidence of dissemination in the tissues examined of Calf 586. (3) Doses of 50 mg. of culture from Calf 520 were inoculated into Calves 602, 614, and 630. In the first animal disseminated tuberculosis of a chronic, but probably slowly progressive type was produced. The second animal died in 33 days with acute tuberculosis of the lungs. The third exhibited acute disseminated tuberculosis.

(3) *In Lemurs, Baboons, and Monkeys.*

(1) *In Lemurs.*—Feeding with 1 mg. of culture of E II (from original material) produced severe disease in Lemur 18 ; and a like dose of the same culture, subcutaneously inoculated, caused acute tuberculosis in Lemur 16. A like subcutaneous dose of E III caused acute tuberculosis in Lemur 14.

(2) *In Baboons.*—Feeding with 1 mg. of culture of E IV (from original material) produced only slight disease in Baboon 74 ; and a like dose of the same culture, subcutaneously inoculated, caused disseminated tuberculosis of retrogressive type in Baboon 72.

(3) *In Monkeys.*—Feeding with 1 mg. of culture of E II (from original material) produced only slight disease in Monkey 186 ; and a similar result was obtained in Monkey 182, fed with 1 mg. of culture of E IV (from original material). In Monkey 208 feeding with 50 mg. of culture of E II (from original material) produced disseminated tuberculosis of a chronic type ; in Monkey 212, fed with a like dose of culture of E II (from Calf 404), only slight and apparently non-progressive lesions were produced ; and in Monkey 202, fed with 60 mg. of culture of E II (from original material), very little evidence of tuberculosis was found. Subcutaneous inoculation of ·1 mg. of E II (from original material) produced in Monkey 184 disseminated lesions of a mild type ; 1 mg. of the same culture produced still milder infection in Monkey 180, but in Monkey 216 a like subcutaneous dose of the same culture caused general dissemination and abundant multiplication of bacilli, but with relatively little tissue destruction. Subcutaneous inoculation of 1 mg. of E II (from Calf 408) caused disseminated tuberculosis of a mild type in Monkey 220. The subcutaneous inoculation of 1 mg. of E IV (from original material) caused progressive tuberculosis of an acute type in Monkey 178, and disseminated tuberculosis of a chronic type in Monkey 218. The subcutaneous inoculation of 10 mg. of E II (from original material) caused progressive disseminated lesions in Monkey 168.

The instances of relatively low virulence of E II and E IV for the monkey may be compared with similar results obtained by certain human lupus viruses, to which I have called attention above.

(4) *In Other Mammals.*

In four rabbits, the lesions produced by the viruses E I, E III, and E V were identical with those caused by highly virulent human, bovine, and porcine viruses. The effects of subcutaneous inoculation were much less severe than those produced by intravenous inoculation. The viruses E II and E IV have produced disseminated disease, but it was of a more chronic character in some cases, *e.g.*, in Rabbits 1082, 1083, and 1080, inoculated subcutaneously with culture from original material.

But in Rabbit 1418, 1 mg., subcutaneously, of E IV (through Calf 502) produced severe disease. Intravenous inoculations of cultures from original material of E II and E IV produced advanced and fatal disease, but the infection was less acute in type than that caused, intravenously, by E I.

In swine, E II in a subcutaneous dose of 10 mg. produced general tuberculosis in one case, but the lesions appeared to be quiescent and contained very few bacilli; in another case, after 50 mg., subcutaneously, of the same virus, the lungs were severely affected and contained very large numbers of bacilli. E III in a subcutaneous dose of 10 mg. produced about as much disease as 50 mg. of E II, but the experiment was terminated at an earlier period (63 days as compared with 174 days). E IV, in a subcutaneous dose of 10 mg., produced the same result as the same dose of E II, and in a dose of 50 mg. has not produced more severe disease.

In guinea-pigs the tissue reaction was the same as that produced by mammalian bacilli of human or porcine origin. All the animals showed disseminated disease, but with less incidence upon the kidneys than upon other organs. The three animals inoculated with E IV are examples of a relatively mild type of infection. The animals inoculated with E II and E III showed more extensive disease, particularly in the liver, but it is noticeable that E III, the more virulent of the two viruses, has not produced infections of more severity than E II.

(5) *In Fowls.*

After intravenous inoculation the bacilli have been generally disseminated and have produced slight irritant effects in the sites where they have been deposited. They showed no signs of multiplication and have not led to the formation of tubercles.

LESIONS PRODUCED BY MAMMALIAN TUBERCLE BACILLI ISOLATED FROM A GNU AND AN ANTELOPE.

These two viruses produced in the rabbit advanced, slowly progressive disease identical in type with that caused by other mammalian viruses possessing relatively low virulence for the bovine.

Calf 510, inoculated subcutaneously with 50 mg. of culture obtained from the antelope, showed no signs of dissemination in the specimens examined.

THE BACTERIOLOGICAL RESULTS WITH MAMMALIAN VIRUSES.

ADDITIONAL OBSERVATIONS ON THE CULTURAL CHARACTERS OF BOVINE VIRUSES.

Since the publication of the Second Interim Report no new bovine viruses have been investigated, but additional experimental animals have been inoculated with the old viruses by Dr. F. Griffith at Walpole Farm, and the characters of the cultures obtained from them have been studied. As a convenient distinction from the term "virus," which is used to designate all the bacilli derived from a spontaneously infected bovine which formed a starting-point of the investigations, these cultures raised from particular animals are termed "strains."

I have examined 31 additional strains, and classified them on the same principle and by the same methods as in my previous Report. They are arranged, according to their abundance of growth, in Grades I, II, III, &c., which form an ascending scale, Grade I being the class containing the cultures which grow least well on the test media. My results, compared with those previously reported, are as follows:—

Virus.—B IV.

No. of strains previously reported—14, viz., 11 in Grade I, 1 in Grade II (Dog 116), 1 in Grade III (Dog 92), and 1 in Grade IV (Dog 18).

No. of new strains—7, viz., 3 in Grade I (2 from Dog 150 and 1 from Rabbit 1899), 3 in Grade II (2 from Dog 116 and 1 from Chimpanzee 22), and 1 in Grade III (Fowl 394).

Virus.—B VII.

One strain previously reported, in Grade II.

Two new strains, both in Grade III (Fowls 442 and 444).

Virus.—B VIII.

One strain previously reported, in Grade II.

Two new strains, both in Grade II (Dogs 142 and 156).

Virus.—B IX.

Number of strains previously reported—14, viz., 13 in Grade III, and 1 in Grade IV (Dog 50).

Number of new strains—8, viz., 2 in Grade II (Monkey 152 and Chimpanzee 18), and 6 in Grade III (3 from Dogs 126 and 136, 2 from Fowls 430 and 432, and 1 from Monkey 126).

Virus.—B XII.

Two strains previously reported, 1 in Grade I and 1 in Grade II.

Two new strains, both in Grade I (Dog 160 and Pony 6).

Virus.—B XXII.

Two strains previously reported, 1 in Grade I and 1 in Grade III (Dog 72).

One new strain, in Grade III. (Dog 126).

Virus.—B XXVI.

One strain previously reported, in Grade II.

Nine new strains—6 in Grade I (Rabbits 1903, 1904, 1908, Parrots 6 and 8, Fowl 516), and 3 in Grade II (Fowls 84 and 450, and Guinea-pig 3331).

Results.

Compared with former results, the new strains do not exhibit any very important differences. Sometimes, though not invariably, a change in the direction of increased luxuriance has been observed after residence in the tissues of a dog. The results on birds, taken as a whole, show very little evidence of change, though in some instances the cultures recovered from birds have grown rather better than previously; their characters have always remained in strict conformity to the mammalian type.

ADDITIONAL OBSERVATIONS ON THE CULTURAL CHARACTERS OF HUMAN VIRUSES.

My observations deal with 15 additional strains from 2 old viruses and 109 strains from 44 new viruses.

(1) *Old Viruses.*

Further investigations carried out by Dr. A. S. Griffith at Blythwood have enabled me to obtain additional cultures.

H 13. A.D.—Three strains were previously reported, 1 in Grade IV (Calf 301), and 2 in Grade I (Rat 15 and Calf 321, each from Calf 301). Six new strains have been classed, 1 in Grade V (Calf 1175 from Calf 301), and 5 in Grade I (2 strains of Calf 1177 from Calf 301, and 3 strains of Calf 1237 from Calf 1175).

H 53. D.H.—Two strains were previously reported, both in Grade III. 9 new strains have been classed, all in Grade III, viz., 8 derived through various animals from the same original human material (a), and 1 derived from the second original human material (b) obtained from the same subject.

Comments.—These results confirm those previously obtained. In the case of H 13. A.D. it has again been shown that cultures of very much lower luxuriance may, by animal passage, be obtained from the culture derived from Calf 301. The classification of H 53. D.H. has been confirmed by 8 additional strains derived, ultimately, from the same original material, and by a strain derived from tissue removed $3\frac{8}{12}$ years subsequently from the same human subject.

(2) *New Viruses.*

In 22 out of the 44 new viruses I have not investigated more than one strain. In each of the remaining 22 more than one strain was examined; some of these latter viruses presented features of special interest, in others the reason for examining more than one strain was mainly to see how far strains presumably identical in character yielded identical features on artificial media. 86 strains belonging to these 22 viruses were examined.

Out of the total 44 new viruses, 2 were classed in Grade I, 3 in Grade II, 1 in Grades II and III, 8 in Grade III, 1 in Grades II and IV, 1 in Grades III and IV, 16 in Grade IV, 7 in Grades IV and V, and 5 in Grade V.

In relation to the original human material the distribution is as follows:—

2. *Grade I.*—H 77. R.L. (Liver and Mesenteric Gland—age $1\frac{1}{12}$ years); H 89. W.I. (Mesenteric Gland and Lung—age 4 years).

3. *Grade II.*—H 100. R.S., H 105. G.S., and H 110. J.B. (all Lupus).

1. *Grades II and III.*—H 85. H.B. (Lupus).

8. *Grade III.*—H 69. F.K. (Mesenteric Gland and Meninges of Brain—age 2 years); H 72. H.C. (Abscess of Rib—age 49 years); H 88. E.L. (Mesenteric Gland—age $8\frac{7}{12}$ years); H 91. H.S., H 101. E.G., H 107. H.H., H 108. H.R., and H 111. S.E. (all Lupus).

1. *Grades II and IV.*—H 90. I.P. (Mesenteric Gland and Retro-peritoneal Gland—age 70 years).

1. *Grades III. and IV.*—H 60. W.B. (3 strains from Bronchial Gland in III., 1 strain from Bronchial Gland and strains from Brain, Mesenteric Gland, and Lung in IV.—age $4\frac{7}{12}$ years).

16. *Grade IV.*—H 67. E.P. (Scapular Abscess—age 14 years); H 68. R.B. (Sacral Abscess—age 69 years); H 70. N.W. (Lung, Cervical Gland, Bronchial Glands, and Mesenteric Glands—age $\frac{10}{12}$ year); H 74. D.S. (Mesenteric Gland, Bronchial Gland, and Spleen—age $\frac{7}{12}$ year); H 76. G.M. (Knee Joint—age 30 years); H 82. G.F. (Mesenteric Glands and Lung—age 4 years); H 84. M.S. (Lupus); H 86. C.P. (Meninges of Brain—age $3\frac{7}{12}$ years); H 87. W.D. (Suprarenal Capsule—age 21 years); H 93. C.D. (Lung and Acne Spot—age $2\frac{3}{12}$ years); H 98. B.R. (Cerebro-Spinal Fluid—age 7 years); H 102. N.H., H 103. N.S., H 106. K.R., H 109. M.W. (all Lupus); H 115. N.G. (Cerebro-Spinal Fluid—age 3 years).

7. *Grades IV and V.*—H 71. L.V. (Lupus—strain (*a*) in IV, (*b*) in V); H 75. A.H. (Peritoneal Fluid in IV, Lung in V—age 7 years); H 78. O.D. (Bronchial Gland and Lung in IV, Meninges of Brain in V—age $\frac{9}{12}$ year); H 79. J.N. (4 strains from Lung in IV, 8 strains from Lung in V—age 24 years); H 80. D.M. (6 strains from Lung in IV, 3 strains from Lung in V—age 33 years); H 81. P.W. (2 strains from Lung in IV, 7 strains from Lung in V—age 33 years); H 99. L.K. (Lupus—strain from one G.P. in IV, from another in V).

5. *Grade V.*—H 83. G.C. (Mesenteric Gland, Bronchial Gland, Meninges of Brain—age $1\frac{9}{12}$ years); H 92. D.N. (Lupus); H 104. E.R. (Lung—age 31 years); H 112. B.B., H 114. A.U. (both Lupus).

THE CULTURAL CHARACTERS OF BACILLI ISOLATED FROM HUMAN SPUTUM.

In addition to the preceding strains Dr. A. S. Griffith has sent to me for examination cultures which he isolated from the sputum of 28 patients. Before collecting the sputum special precautions were taken to avoid the risk of contamination by living tubercle bacilli of animal origin communicated through food ingested. These viruses were numbered H 118—H 145. Twenty-three of them grew luxuriantly on my usual test media; in my notes I have classed them in Grade V in all but one instance, when I described the growth as being equivalent to the best of Grade IV.

Special attention may be called to the remaining five viruses. I found H 118. F.C. to be typical of the poorer growths in Grade III. With H 122. F.P. the growths on broth and glycerin-agar were typical of Grade V, but on potato (three trials) the growths were no better than those of Grade III. H 127. R.R. and H 128. D.D. were typical of Grade I. With H 133. P.K. the growths on broth and potato were equivalent to those of Grade IV, but on glycerin-agar (three trials) growths were no better than the poorer specimens in Grade III.

THE CULTURAL CHARACTERS OF SWINE VIRUSES.

Fifty-eight swine viruses, derived from spontaneously infected animals, have been investigated. 51 of these, derived in all except 12 cases from submaxillary lymphatic glands, were found to be identical in every respect with ordinary bovine viruses, and are classed in one or other of Grades I, II, and III. These call for no further comment, but attention may be called to the remaining seven.

P IV.

The strains from the original material (submaxillary lymphatic gland) of this virus and from Calf 312 grew abundantly and were classed in Grade V. But the strain from Rabbit 557, inoculated with culture from Calf 312, grew less well and was classed in Grade III; and the growths from two strains of Rabbit 556, also inoculated with culture from Calf 312, were still poorer, and were classed in Grade II.

P XLI.

The strains from original material (submaxillary lymphatic gland) and Pig 166 were classed in Grade IV, the strain from Pig 202 in Grade V.

P II, P III, P XXI, P XXVII, P XLIV.

The strains from the original material (submaxillary lymphatic glands) of these five viruses presented the typical characters of the avian bacillus; but the strain of P II from the spleen of Monkey 118, and a strain of P III derived from Calf 282 were mammalian in character and were classed in Grade III. It does not appear to be possible to draw any definite conclusions from these last two exceptional results.

THE CULTURAL CHARACTERS OF EQUINE VIRUSES.

The five viruses examined, which were obtained from spontaneously infected horses, resembled in cultural growth the ordinary bacillus isolated from the bovine animal. The first three grew very badly in early generations, presenting more difficulty in this respect than even very poorly growing bovine viruses; but after prolonged subculture they showed marked improvement, though not in any case surpassing the maximum luxuriance of Grade III. Strains passed through other species of animals, including the fowl, showed no important change in cultural characters.

THE CULTURAL CHARACTERS OF THREE OTHER MAMMALIAN VIRUSES.

A virus from a spontaneously infected cat grew fairly well, though not better than some bovine viruses. A virus from a spontaneously infected antelope grew as well as many slightly virulent human viruses; and a virus from a spontaneously infected gnu was extremely luxuriant in its growth.

COMPARISON OF THE VIRUSES DERIVED FROM MAN WITH THOSE DERIVED FROM OTHER MAMMALS.

In my last Report I showed that both as regards their bacteriological characteristics and their effects upon the tissues of experimental animals, the viruses derived from bovines were identical with certain of the viruses derived from human subjects. As no additional viruses from the bovine have been investigated, there is nothing to add to this statement, in so far as it relates to the characteristics of bovine viruses.

But to the previously recorded human viruses my investigations now show that there are to be added, as histologically and culturally identical with the bovine viruses, the human viruses H 77. R.L., H 88. E.L., H 89. W.I., H 110. J.B., and, culturally, the two viruses H 127. R.R. and H 128. D.D. which were derived from human sputum.

Out of the fifty-eight viruses obtained from spontaneously infected swine I have found that fifty are, culturally and histologically in so far as my investigations go, identical with all those above mentioned human viruses which have been found identical with the bovine viruses.

The remaining eight swine viruses differed from the above. Three of them, *viz.*, P IV, P XLI, and P XLVI, were found to resemble pathogenically certain human viruses. The remaining five were radically different from all the human viruses ; they exhibited the characteristics of the avian tubercle bacillus.

Five equine viruses have been examined. Three of them resembled those human viruses which were found to be identical with the bovine viruses. The remaining two, E II and E IV, differed from these three but resembled certain other human viruses, notably the three human viruses H 100. R.S., H 105. G.S., and H 108. H.R., which were obtained from cases of lupus.

A virus from a spontaneously infected cat resembled culturally those human viruses which were found to be identical with the bovine viruses.

A virus from a spontaneously infected antelope and also a virus from a spontaneously infected gnu proved identical with those human viruses which differed markedly from the bovine viruses by the possession of greater cultural luxuriance and lower pathogenicity for calves and rabbits.

The general result, therefore, in comparing the animal viruses with the human viruses, is that, with exception of the avian viruses noted above, every variety of virus obtained from the lower mammals has also been found in man.

THE RANGE OF VARIETY IN THE VIRUSES EXAMINED.

In reporting on the range of variety in the viruses which I have examined, it is convenient to begin with the two ends of the scale.

First there are the viruses which have consistently produced in the bovine and the rabbit lesions typical of highly acute tuberculosis. These viruses, though differing among themselves in their degrees of cultural luxuriance, are all comprised within my Grades I to III.

At the other end of the scale there are the viruses which, when tested appropriately, have consistently produced in the bovine and the rabbit lesions typical of less acute, chronic, or retrogressive tuberculosis. These viruses are of greater cultural luxuriance than the former, being classed by me in Grades IV and V.

Then there are viruses which, for one reason or another, occupy an intermediate position in the scale. To these I now refer in detail, arranging them under headings which indicate what appears to me the reason for placing the viruses in question in an intermediate position.

VIRUSES EXHIBITING SOME ANOMALIES.

(1) *Dissimilarity of Bacilli from Different Parts of the same Human Body.*

H 60. W.B.—The main facts on which I am able to report regarding this virus are as follows :—The cultures which I investigated were derived from (1) a bronchial gland, (2) the lungs, (3) a mesenteric gland, and (4) the brain of the human subject. The culture from (1) (fifth generation), through Guinea-pig 1825, was classed amongst the best growths of Grade III ; Calf 1107 was inoculated with this culture ; cultures from the prescapular and a portal gland of Calf 1107 were classed amongst the average growths of Grade III. The cultures from (2), (3), and (4) grew better and were classed in Grade IV, (2) being amongst the average and (3) and (4) amongst the best growths in this Grade. Experimentally, as I find from the material sent to me for examination, (1) proved to be of high virulence, whereas in two calves inoculated respectively with (2) and (4) there was evidence of much lower virulence. I have examined the tissues of four calves representing the effects of (1) and found them all to exhibit severe infection, whereas in two calves taken as representative of the effects of the other three strains the type of disease is very much milder. These results indicate that the bacilli derived from the human bronchial gland differed, both culturally and in virulence, from those derived from the lungs and brain of the same subject. I may also call attention to a minor point regarding (1). I have examined a culture from Rabbit 1228, which had been inoculated with this strain after it had been subcultured for many generations on glycerinated media; and I have found that, apparently owing to the influence of the glycerin, there is a slight increase of luxuriance, the culture being classed amongst the poorer growths of Grade IV.

H 69. F.K.—Cultures were examined (1) from the mesenteric gland and (2) the meninges of the human subject, and were both classed amongst the best growths of Grade III. The tissues of Calf 1249, after inoculation, when 13 weeks old, with 50 mg. of (1), exhibited relatively mild disease; but the tissues of Calf 1267, inoculated, when 17 weeks old, with a like dose of (2), exhibited typically acute infection. It therefore appears that the bacilli derived from the human meninges were of somewhat higher virulence, in this particular instance, than those derived from the mesenteric gland.

H 90. I.P.—Cultures (1) from a retro-peritoneal gland (direct) and (2) from a mesenteric gland (through Guinea-pig 2522) of the human subject were both classed in Grade IV, the former growing rather better than the latter. In the tissues of Calves 1383 and 1471, inoculated with 50 mg. and 80 mg. respectively of culture from (1), the lesions are mild and retrogressive in character; but cultures from Calves 1383 and 1471 grew much more poorly, being classed in Grade II, and in Calves 1489 and 1493, inoculated with cultures obtained respectively from Calves 1383 and 1471, the lesions show highly acute infection.

(2) *Initial Irregularity of Bacilli from the same Original Human Site.*

H 49. T.C.—I have investigated, and already reported upon, material from three strains of this virus, which was derived from the mesenteric gland of a young adult. Strain (1) (direct from original material) was classed by me in Grade II; strain (2) (Calf 787, which had been inoculated with 1) was placed in Grade III; and strain (3) (Calf 797, which had been inoculated with 1) was placed in Grade II. Calves 787 and 797 were inoculated each with 50 mg. of strain (1) when the strain had been two months in cultivation; the first animal was fatally infected in 81 days, the latter was killed, with only a slight amount of disease, in 83 days. After the same strain had been 7 months in cultivation it was inoculated in doses of 50 mg. into Calves 957 and 959; both were fatally infected, the former in 61 and the latter in 53 days. After the same strain had been 14 months in cultivation, it was inoculated into Calf 1097, in a dose of 50 mg., and produced in this animal only a slight amount of disease, as reported by Dr. Cobbett. The virulence of strain (3) was also tested upon bovines and proved highly virulent in doses of both 50 mg. and 10 mg. Further investigations of strain (1) in my laboratory have shown that when it had been two years in cultivation it produced, in a dose of 50 mg., slowly progressive tuberculosis of an intermediate or chronic type in Calf 1213; and that a tissue emulsion from this animal produced in Calf 1263 disseminated caseous tubercles of retrogressive type. Nevertheless a culture from Calf 1263, in a dose of 50 mg., produced acute tuberculosis in Calf 1347. Another culture from strain (1) (through G.P. 2918) produced, in a dose of 48 mg., acute tuberculosis in Calf 1501. My work on this virus, therefore, illustrates irregularities in the pathogenic effects of the bacilli derived from the original human mesenteric gland.

H 53. D.H.—Material (scrapings of skin) from this case (human lupus) was received by my colleagues on two occasions, (a) on January 17, 1905, and (b) on August 17, 1908. I have examined cultures of 10 strains from (a) and of 1 strain from (b), and have classed them all in Grade III. Cultures from (a) have failed to produce severe disease in calves; but cultures from (b) produced acute infection in Calves 1507 and 1545, though in Calf 1535, inoculated with the same material and the same dose (50 mg.) as Calf 1545, I have found only slight evidence of disease. Taking the results as a whole, the bacilli present in the infected area of skin appear, from the specimens which I have examined, to have possessed a distinctly higher initial experimental virulence in the year 1908 than in the year 1905.

H 100. R.S. — Cultures from the original material (human lupus), in doses of 50 mg., produced, as shown by histological examination, typically acute tuberculosis in Calf 1523, but only chronic tuberculosis in Calf 1419 (aged nine weeks when inoculated); and 100 mg. of original culture produced only chronic tuberculosis in Calf 1409 (aged 24 weeks when inoculated). The cultural characters of this strain were classed by me amongst the best growths of Grade II.

(3) *Histological Evidence of Increased Virulence, with Bacteriological Evidence of Diminished Cultural Luxuriance.*

H 13. A.D.—In my previous Report I dealt with this virus (human bronchial glands and spleen). In the earlier experiments, carried out by Dr. Cobbett, the virus was of low virulence; but after animal passage severe disease was produced suddenly in Calf 301. The culture from this animal was classed in Grade IV. This culture, 15 months after isolation, was inoculated, in doses of 50 mg., into Calves 761 and 765, and produced in these animals, as reported by Dr. Cobbett, only slight disease. But in Calf 321, inoculated with emulsion of the tissues of Calf 301, acute disease had been produced, and the culture from Calf 321 was classed in Grade I. This last culture produced acute disease in Calf 825. Later experiments, by Dr. A. S. Griffith, have yielded me material showing that, when the strain from Calf 301 had been additional 23 months in artificial cultivation, doses of 50 mg. and 100 mg. of culture produced no more than chronic disseminated tubercles in the tissues of Calves 1175, 1177, and 1207. In Calf 1237, inoculated with a tissue emulsion from Calf 1175, the lesions were much more extensive but were not, in the specimens which I examined, equal in severity to those found in highly acute infections. A culture from Calf 1175 I have classed in Grade V; but two cultures from different organs of Calf 1177 and three cultures from different organs of Calf 1237 have been classed by me in Grade I.

H 16. J.H.—This virus has been dealt with in my former Report.

P IV.—The culture obtained from the original material (submaxillary lymphatic gland of a pig) was classed in Grade V, and produced disease which is histologically of intermediate severity in Calf 312, but only slight disease in Calf 314. The culture from Calf 312 was also classed in Grade V; it produced only slight disease in two calves, and also in Rabbits 556 and 557. But a culture from Rabbit 557 was classed in Grade III; and cultures from two organs of Rabbit 556 were each classed in Grade II.

(4) *Cultural Characters compatible with High Virulence; Histological Evidence of Initial Low Virulence followed by an Increase.*

H 100. R.S.—This virus (original material) was classed in Grade II. Two cultures from the original material (human lupus) produced only mild disease in Calves 1409 (100 mg.) and 1419 (50 mg.). But the cultures from these two calves produced, in doses of 50 mg., acute tuberculosis in Calves 1511, 1517, and 1543.

H 105. G.S.—The culture from the original material (human lupus) was classed in Grade II, but produced only mild disseminated disease in Calves 1405 and 1449, after doses, respectively, of 92 mg. and 48 mg. But the cultures from these two calves, in doses of 50 mg., produced acute disease in Calves 1521 and 1527.

H 107. H.H.—The culture from the original material (human lupus) was classed in Grade III, but, in doses of 50 and 80 mg., produced only slight disease in Calves 1453 and 1497. The culture (50 mg.) from Calf 1497 produced only mild disease in Calf 1549; but the culture from Calf 1453, whilst producing mild disease in Calf 1505 (with 100 mg.) and in Calf 1513 (with 50 mg.), produced acute disease in Calf 1519 (with 50 mg.).

H 108. H.R.—The culture from the original material (human lupus) was classed in Grade III, but (in doses of 50 and 88 mg.) it produced remarkably little disease in Calves 1417 and 1421. But 50 mg. of culture from Calf 1417 produced very acute disease in Calf 1539; and 50 mg. of culture from Calf 1539 produced typically acute infection in Calf 1509.

E II.—The culture first investigated by me was derived from a guinea-pig inoculated with original material (a mesenteric gland of a horse). For several generations the culture grew very badly (Grade I); later generations grew much better and attained to Grade III, but never exceeded this degree of cultural luxuriance. This culture produced a moderate amount of disease in (1) Calf 406 (10 mg.), but only slight disease in (2) Calf 404 (10 mg.), in (3) Calf 504 (50 mg.), and in (4) Calf 506 (50 mg.). From (1), a culture was raised and passed into Calf 596 (50 mg.), where it produced slowly progressive tuberculosis of the lungs; a culture from Calf 596 was classed in Grade I; it produced acute tuberculosis in Calf 638 (50 mg.). From (2), a culture was raised and was classed in Grade I; this culture was passed into Calf 590, but the animal died prematurely of intercurrent disease; a culture from Calf 590 was classed in Grade I; it produced, according to Dr. F. Griffith's report, only slight disease in Calf 618 (50 mg.). From (3), cultures were raised from the prescapular gland and lung and were both classed in Grade I; 50 mg. of the culture from the prescapular gland produced acute tuberculosis in Calf 626; 50 mg. of the lung culture produced mild, retrogressive infection in Calf 578 but typically acute infection in Calf 580. From (4), a culture was obtained and was classed in Grade I; this culture produced moderately severe infection in Calf 628 (50 mg.); a culture from Calf 628 produced acute disease in Calf 646 (50 mg.), as reported by Dr. F. Griffith.

E IV.—The first culture investigated by me was obtained from a guinea-pig which had been inoculated with the original material (spleen of a horse). It was classed in Grade III. It produced only slight disease in Calf 410 (10 mg.), in Calf 502 (50 mg.), and in Calf 620 (50 mg.), but in Calf 498 (50 mg.), there was acute tuberculosis of the lungs, suggestive of intravenous inoculation. From another calf (408), in which Dr. Griffith found only slight disease, a culture was obtained and was classed in Grade III; it produced a moderate amount of disease in Calf 582 (50 mg.); no increase of virulence for calves was obtained in subsequent passage experiments from this culture. From Calf 498 cultures were obtained (bronchial gland and lung) and were classed in Grade III; 50 mg. of the lung culture failed to produce any evidence of dissemination in the tissues examined of Calf 586; no increase of virulence for calves was obtained in subsequent passage experiments from the cultures of Calf 498. From Calf 502 a culture was obtained and was classed in Grade III. Doses of 50 mg. of culture from Calf 502 were inoculated into Calves 602, 614, and 630; in the first animal disseminated tuberculosis of a chronic but probably slowly progressive type was produced; the second animal died in 33 days with acute tuberculosis of the lungs; the third exhibited acute disseminated tuberculosis. A culture from Calf 602 produced acute tuberculosis in Calf 650, as reported by Dr. Griffith. From Calf 616, another calf, inoculated with the first culture, in which Dr. Griffith found only slight disease, a culture was obtained and was classed in Grade II.

(5) *Intermediate Viruses in Grade III.*

In classifying my cultures I have found only a slight difference between some of the best growths in Grade III and some of the poorest growths in Grade IV. The following viruses, classed by me in Grade III, yielded histological evidence of low virulence, and in this respect resembled the better growing viruses of Grades IV and V.

H 72. H.C.—This virus (abscess from a human rib) was classed by me amongst the best growths of Grade III. It proved to be of relatively low virulence for the bovine.

H 85. H.B.—The culture obtained through a guinea-pig from the original material (human lupus) was classed by me in Grade III. Cultures through Chimpanzee 11 were also classed in Grade III. A culture from Monkey 121 was classed in Grade II. The virus proved to be of low virulence for the bovine.

H 91. H.S.—This virus (human lupus) was classed by me in Grade III. It was found by Dr. A. S. Griffith to be of low virulence for the bovine (one animal) and rabbits.

H 101. E.G.—This virus (human lupus) was classed by me amongst the best growths of Grade III. It was found by Dr. A. S. Griffith to be of low virulence for the bovine (one animal) and rabbits.

H 111. S.E.—This virus (human lupus) was classed by me in Grade III. It proved to be of relatively low virulence for two bovines in doses of 50 mg.

P XLVI.—This virus (submaxillary lymphatic gland of a pig) was classed by me amongst the best growths of Grade III. It was found by Dr. F. Griffith to be of low virulence for the bovine in a dose of 50 mg. and a dose of 75 mg.

THE RESULTS WITH AVIAN TUBERCLE BACILLI.

THE HISTOLOGY OF LESIONS PRODUCED BY AVIAN VIRUSES.

No histological difference has been found in the pathogenicity of the various viruses, exhibiting the cultural characters of the avian bacillus, which I have investigated.

(1) *In Fowls and Parrots.*

After intravenous inoculation of avian bacilli in fowls, severe and rapidly fatal infection is produced. The brunt of the disease falls on the liver and spleen ; and in these organs there is an extremely copious multiplication of bacilli. The lungs and kidneys are much less susceptible. Many bacilli lodge in the lungs, and there multiply into colonies ; but they produce very little tissue damage, much less than has been found in several cases after intravenous inoculation of fowls with highly virulent mammalian bacilli. In the kidneys also, avian bacilli may be detained, though in much smaller numbers than in the lungs ; when detained, they frequently grow into small colonies but produce very little tissue damage.

After feeding in fowls, severe disease may be produced by the avian bacillus ; but it is slower in development than after intravenous inoculation, and is associated with caseous tubercles surrounded by giant cells, or multinucleate cell masses, and a fibrous periphery. The incidence of the disease on different organs is the same as after intravenous inoculation, the liver and spleen being most severely affected.

After subcutaneous inoculation of avian bacilli in fowls, the general results are similar to those obtained by feeding ; but, in addition to the liver and spleen, the lungs may exhibit caseous nodules.

Intraperitoneal inoculation in fowls may produce similar effects to those of intravenous inoculation in the liver and spleen, but seems to be less certain in its results.

Dead avian bacilli, intravenously inoculated, exert a toxic effect upon the fowl, and produce more damage in the liver and spleen than in other organs.

The specimens of parrots examined show that these birds are susceptible to the avian bacillus, administered intravenously, by feeding, or by intramuscular inoculation, and that caseous lesions are readily produced.

(2) *In Calves.*

After intravenous inoculation, rapidly fatal infection is usually produced in young calves by the avian bacillus. It is associated with general dissemination and very abundant multiplication of the bacilli ; but there is relatively little tissue destruction, and there is an absence of tubercle formation. These results resemble the condition found in young calves which have rapidly succumbed to the intravenous inoculation of mammalian bacilli possessing low virulence for the bovine when inoculated subcutaneously. But the avian bacilli multiply even more profusely, and often grow into the solid colonies and rosettes which are characteristic of this bacillus.

Apart from other toxic effects which may have been associated with the profuse growth and dissemination of the avian bacilli, the anatomical condition of the lungs of the calves seems to have been sufficient to account for their death. To the naked eye the specimens presented extreme oedema with patches of complete consolidation, some in the red and some in the grey stage. These grey patches were sometimes surrounded by areas in the red stage, but, as my microscopic examinations show, they were not caseous tubercles.

Though the total amount of gross tissue destruction was small, as compared with the acute disease produced by highly virulent mammalian bacilli after subcutaneous inoculation of a large dose, microscopic examination showed, particularly in the liver, that the tissue cells were not left in a normal condition. The livers of Calves 524 and 544, for example, when stained appropriately, showed extensive damage to the parenchymatous cells.

The subcutaneous inoculation of 50 mg. of avian culture failed to produce more than a mild degree of general dissemination, and in some cases the disease appears to have been almost limited to the nearest glands. The disseminated lesions which have been found were usually of a definitely retrogressive character, and bacilli, when present in them, were rarely numerous. Caseation and giant cell formation were not uncommon, and the general structure of some of the lesions was indistinguishable from that of chronic tubercles produced by mammalian bacilli.

(3) *In Rabbits.*

(1) *Intravenous Inoculation.*—Dead avian bacilli, even in so large a dose as 50 mg., were rapidly eliminated ; traces of their presence were often found in small foci of irritation, more particularly in the lungs, and some of these amounted to small, slightly caseous sites indistinguishable from tubercles produced by mammalian bacilli. The inoculation of a very small dose of living bacilli caused disseminated tuberculosis of a chronic and relatively mild type, the lesions being discrete, circumscribed, and centrally caseous. When a larger dose, *e.g.*, 1 mg., was inoculated, rapidly fatal disease was produced ; the bacilli became disseminated throughout the body and grew very abundantly, forming numerous colonies and rosettes ; the amount of tissue destruction on the whole was relatively slight, though severe irritant effects were sometimes observed in the lungs ; the bacilli showed a marked tendency to intracellular growth, and, though giant cells were formed, tubercles of the discrete, circumscribed, caseous type were rare.

(2) *Feeding.*—This proved a much less severe mode of infection. Disseminated lesions were sometimes found, but they were small and of the chronic, caseous type.

(3) *Subcutaneous Inoculation.*—Disseminated caseous lesions were frequently produced, and were sometimes extensive and progressive, though usually containing relatively few bacilli.

(4) *Intraperitoneal Inoculation.*—General dissemination and abundant multiplication of bacilli resulted, the effects being similar to those produced by intravenous inoculation.

(4) *In Monkeys.*

Intravenous inoculations of avian bacilli have usually proved fatal to these animals. In the tissues of one, inoculated with 1 mg. of culture, bacilli were scanty and only a few lesions were definitely attributable to their presence. But in the animals inoculated with larger doses (10 to 100 mg.) the bacilli multiplied very abundantly and often grew into typical rosettes, particularly in the liver and spleen ; the amount of tissue damage was relatively small, and there was no formation of tubercles. The general features of the tissues resembled those produced in rabbits after intravenous inoculation with avian bacilli. In the monkey, as in the rabbit and fowl, the liver and spleen were found to be more favourable sites than the lungs for the profuse multiplication of the avian bacillus.

With one exception, Monkey 118, monkeys proved highly resistant to subcutaneous inoculation of avian viruses.

(5) *In Swine.*

Intravenous inoculation of avian bacilli produced general dissemination and enormous multiplication of bacilli, with relatively little tissue destruction.

Intraperitoneal inoculation, in one case, produced a similar result, but with rather less copious multiplication of bacilli.

The subcutaneous inoculation of 50 mg. of culture produced in Pig 180 general dissemination with relatively little tissue destruction ; the bacilli were not particularly numerous except in a mesenteric gland, where they were very abundant and had formed typical rosettes. But after an identical experiment on Pig 264 neither lesions nor bacilli were found in the tissues examined.

Feeding with 100 mg. produced in Pig 178 general dissemination and enormous multiplication of bacilli, the result resembling the effect of an intravenous inoculation. But feeding with large doses, 7 whole cultures in the one case and 50 mg. in the other, produced very little evidence of dissemination in Pigs 110 and 176.

(6) *In Guinea-pigs.*

The tissues of these animals, of which seven were examined histologically, proved highly resistant to avian viruses, both after subcutaneous and after intraperitoneal inoculation. There was frequently evidence of dissemination; but the lesions, though sometimes caseous, were not, on the whole, typical of tuberculosis, and the bacilli generally seemed unable to multiply or retain their vitality. But in some cases inoculation was followed by death at a comparatively early period; and the evidence of irritant or necrotic change in the liver suggested that the bacilli may have exerted a toxic effect.

(7) *In Goats.*

Intravenous inoculation of avian viruses produced death with general dissemination and abundant multiplication of bacilli, but with no tubercle formation and very little tissue damage.

After subcutaneous inoculation the results were irregular. After 50 mg. no evidence of dissemination was found in the specimens of Goat 42; in Goat 36, after 10 mg., some chronic, caseous tubercles were present in the lungs; and in Goat 48, after 50 mg., there was general dissemination and abundant multiplication of bacilli. The lungs of this last animal contained large, progressive, caseous lesions, but in the other organs the amount of tissue destruction was small and there were no tubercles; the bacilli formed typical rosettes.

(8) *In other Mammals.*

Ponies.—After subcutaneous inoculation and after feeding there was very little evidence of dissemination. Fifteen days after intravenous inoculation with 10 mg. Pony 32 showed general dissemination of bacilli in small numbers; but the bacilli showed no sign of multiplication or of producing progressive disease.

Dogs.—After a subcutaneous dose of 25 mg. there were a few slight, retrogressive lesions in the lungs and liver of Dog 122; bacilli were rare. After an intravenous dose of 40 mg. the bacilli almost entirely disappeared from Dog 162, and left behind only minute, retrogressive lesions.

Cat 62.—In this animal, after an intraperitoneal dose of 1 mg., the tissues were swarming with bacilli, which had produced very little tissue damage.

Rats and Mice.—In many cases the bacilli have multiplied very abundantly in the tissues of these animals, but without the production of tubercles.

THE CULTURAL CHARACTERS OF AVIAN VIRUSES.

All the cultures raised from original avian material, as well as the avian viruses from swine, were practically identical, presenting in common the characteristics of:—very poor growth on pure serum; copious growth on glycerinated serum; refusal to spread from a sloping surface of glycerinated serum on to the surface of broth, but the formation of powdery growth at the bottom of the broth; copious, pale, slimy growth on glycerin-agar; abundant, pigmented growth on potato; in films examined microscopically, a free separation of the bacilli (to be contrasted with the glueing together of mammalian bacilli), a marked prevalence of very short forms in some cultures, and a marked pleomorphism, with not infrequent branching, in others.

Four strains, originally obtained from birds but recovered after residence of the bacilli in mammals, presented the mammalian characters of Grades II and III.

GENERAL CONCLUSIONS.

In the present Report I have extended my range of observations on human and animal tuberculosis. Putting together the work contained in this and in my previous Report, and referring only to tuberculosis caused by the mammalian bacillus, I have investigated, either histologically or culturally and in the great majority of cases by both methods, the viruses derived from 221 different cases of the spontaneously contracted disease. These consist of 30 viruses obtained from bovines, 130 from human beings, 53 from swine, and eight from other animals. The bacteriological part of the investigation deals with the cultural characters of bacilli obtained directly from the original material and also with the cultural characters of the bacilli recovered after residence in experimental animals. It comprises a study, on various differential media, of each of 447 strains of bacilli, 108 of these strains being ultimately derived from bovines, 250 from human beings, 58 from swine, and 30 from other animals. The histological part of the investigation deals with the tissues of 777 animals experimentally infected, viz., 236 animals infected with bovine viruses, 409 with human viruses, 50 with swine viruses, and 82 with viruses derived from other animals. The animals yielding cultures for the bacteriological work and tissues for histological investigation were:—bovines, rabbits, rhesus monkeys, chimpanzees, baboons, lemurs, swine, guinea-pigs, goats, dogs, cats, rats, mice, horses, fowls, and parrots.

I have also investigated the bacteriological characteristics of various strains of avian tubercle bacilli, and have studied the histological changes produced by these bacilli upon the tissues of birds and various mammals.

I have thus had the opportunity for comparing tuberculosis caused by the mammalian bacillus with tuberculosis caused by the avian bacillus.

My observations lead me to the following conclusions:—

1. Comparing mammalian tubercle bacilli of known human origin with mammalian tubercle bacilli of known origin from spontaneous disease in other animals, I find that in every instance the bacilli of origin from animals other than man are identical culturally, microscopically, and in their effects upon the tissues of experimental animals, with bacilli from certain cases of tuberculosis in the human subject.

2. Referring to the anatomical origins of the human viruses which I have investigated, I call attention to the fact that in all parts of the human body, and in human sputum, collected with due precautions to avoid contamination through food, tubercle bacilli have been found which culturally and in histological effects are identical in every respect with the tubercle bacillus commonly present in the bodies of tuberculous cattle and swine.

3. I have found that many of the viruses of human origin grow with greater cultural luxuriance and produce less histological damage in the organs of certain experimental animals than the bacilli commonly obtained from cattle and swine.

4. In the majority of cases my examinations of the cultures and the animal tissues which I have received at different stages during a protracted series of experiments with the same virus have shown that the original characteristics of the virus were remarkably stable.

5. Hence, to such extent as it may be supposed that such series of experiments are comparable to what takes place in a human body infected with tubercle bacilli, my results support the view that changes in the characteristics of a virus do not readily or rapidly take place in the human body.

6. But I have, in addition, examined certain other viruses which, in respect of the details on which I have reported, hold an intermediate position between the viruses usually obtained from human sources, and those of common animal origin.

7. Owing to the existence of these intermediate viruses, I find it impossible to class all the strains of mammalian tubercle bacilli which I have investigated as belonging to one of two stable types.

8. Another significant feature of these intermediate viruses is that they serve to bridge the apparent gap between the viruses, on the one hand, which produce acute tissue destruction in the bovine and rabbit, and are of relatively scanty growth on culture media, and those viruses, on the other hand, which produce less tissue damage in the bovine and rabbit and grow more luxuriantly on artificial media.

9. I find that underlying all the mammalian viruses which I have investigated there is an essential unity of characteristics, the differences observed being differences of degree but not of kind. On artificial culture media they all grow in the same way, though they differ quantitatively in the amount of growth yielded. In the tissues of suitable experimental animals they all produce lesions histologically characteristic of mammalian tuberculosis, though they differ in the intensity of the tissue changes which they set up under similar experimental conditions.

10. Comparing the mammalian with the avian viruses, I find that there are differences not merely of degree but of kind.

11. On appropriate culture media the growth of the avian bacillus can be distinguished without hesitation from the growth of the mammalian bacillus.

12. Comparing the results of similar experiments performed under suitable conditions upon suitable animals, I find that microscopic examination of the tissues unmistakably distinguishes infections due to avian bacilli from infections due to mammalian bacilli.

13. It is, however, not possible in the tissues of all animals, under all experimental conditions, to distinguish with certainty under the microscope lesions due to avian bacilli from lesions due to mammalian bacilli. It is sometimes found that in the tissues of mammals the avian bacillus produces tubercles of a chronic type which are indistinguishable from tubercles produced by mammalian bacilli.

PART II.

COMPARATIVE HISTOLOGY.

A.—ADDITIONAL OBSERVATIONS ON LESIONS PRODUCED BY BOVINE VIRUSES.

I.—IMMUNITY EXPERIMENTS ON BOVINES.

(*a*) TESTS ON VACCINATED CALVES.

In these experiments, made by Drs. A. S. and F. GRIFFITH, calves were vaccinated either (1) with a small dose of bovine bacilli, or (2) with larger amounts of human bacilli possessing relatively low virulence for the bovine; the resistance of the vaccinated animals was then tested by subcutaneous inoculation with 50 mg. of culture of the bovine virus B XXVI.

(1.)

VIRUS—B XXVI.

Animal Inoculated.—Calf 414.

Calf 414 was inoculated subcutaneously (1) with ·001 mg. of culture of B XXVI, (2) 51 days afterwards with 50 mg. of culture of the same virus. The animal died 93 days after the second inoculation.

Lungs.

Specimens from two portions of the lungs have been examined. They show patches of oedema and capillary congestion, but no tubercle bacilli and no lesions in the least suggestive of tuberculosis.

Liver.

There is one doubtful lesion containing many fibroblasts, but neither definite tubercles nor tubercle bacilli have been found.

Kidneys and Spleen.

No tubercle bacilli or lesions suggestive of tuberculosis are present in the specimens examined.

Prescapular Gland.

The specimen contains advanced caseo-calcareous lesions with giant cells. Tubercle bacilli are scanty.

SUMMARY.

In the specimens examined there is no evidence that bacilli have been disseminated beyond the nearest glands, and there is no indication of the cause of death.

(2.)

VIRUS—B XXVI.

Animal Inoculated.—Calf 416.

Calf 416 was inoculated subcutaneously (1) with ·001 mg. of culture of B XXVI and (2) 52 days subsequently with 50 mg. of culture of the same virus. The animal was killed when in good health 89 days after the second inoculation.

Lungs.

The tissue contains several small consolidated patches which are slightly caseous and contain some giant cells. They have not a well marked boundary layer but are irregularly infiltrated with lymphocytes, leucocytes, and plasma cells. Bacilli are scanty. The lesions cannot be definitely pronounced retrogressive, but the rarity of bacilli and the absence of any extensive tissue destruction are indications that they are not actively progressive. No fibrin is present.

Liver, Kidneys, and Spleen.

No lesions or bacilli have been found in the specimens examined.

Mediastinal Gland.

Caseous tubercles are present. Some of them have a well defined fibrous margin and are commencing to calcify. Bacilli are scanty.

SUMMARY.

Tubercles are present in the lungs and mediastinal gland but do not appear to be progressive. None have been found in the other tissues examined.

(3.)

VIRUSES—
H 54. C.W.
H 8. S.C.
B XXVI.

Animal Inoculated.—Calf 923.

Calf 923 received the following subcutaneous inoculations :—(1) 10 mg. of H 54. C.W. ; (2) 181 days afterwards, 50 mg. of H 8. S.C. ; (3) 244 days after the first inoculation, 50 mg. of B XXVI. The animal died 36 days after the last inoculation.

Lungs.

The tissue is in a condition of patchy, tubercular pneumonia. The caseating areas, though large and irregular, are very conspicuously surrounded by and permeated with plasma cells and lymphocytes. There is also an abundant leucocytosis. Bacilli are moderately numerous, but nowhere occur in large groups ; many of them are notably long. Though the disease is severe, there is a well-marked effort at resistance.

Liver.

In the specimen examined only one tubercle has been found. This is very minute, slightly caseous, and contains two bacilli.

Kidneys.

No lesions or bacilli have been found.

Mediastinal Gland.

Tubercle bacilli are scattered, singly, in many parts of the tissue. There are several caseous foci and there is a tendency to fibroid transformation in the tissue surrounding these areas.

SUMMARY.

The lungs are severely affected, though they show some effort at conservative change. There is relatively little disease in the other tissues examined.

(4.)

VIRUSES—
H 54. C.W.
H 8. S.C.
B XXVI.

Animal Inoculated.—Calf 925.

Calf 925 received the following subcutaneous inoculations :—(1) 10 mg. of culture of H 54. C.W. ; (2) 181 days later, 40 mg. of culture of H 8. S.C. ; (3) 244 days after the first inoculation, 50 mg. of culture of B XXVI. The animal was killed when in good health 90 days after the last inoculation.

Specimens have been examined of two portions of lung, liver, and kidney, and also one portion of the spleen and a mesenteric gland. No tubercles or tubercle bacilli have been found.

SUMMARY.

The animal appears to have been completely immunised.

(5.)

VIRUSES—
H 8. S.C.
B XXVI.

Animal Inoculated.—Calf 1017.

Calf 1017 was first inoculated intravenously with 10 mg. of culture of H 8. S.C., and 63 days afterwards was inoculated subcutaneously with 50 mg. of culture of B XXVI. The animal was killed 95 days after the second inoculation.

Lungs, Liver, Kidneys, Spleen.

No tubercles or tubercle bacilli have been found in the lungs, liver, kidneys (2 specimens of each tissue) or in the spleen (one specimen).

Cervical Gland.

There is a caseo-calcareous tubercle surrounded by a broad fibrous zone and containing many giant cells. Bacilli are present in it but are very rare.

SUMMARY.

Except the cervical gland none of the tissues examined show evidence of tuberculosis.

(6.)

VIRUSES—{H 8. S.C.
B XXVI.

Animal Inoculated.—Calf 1023.

Calf 1023 was first inoculated subcutaneously with 50 mg. of culture of H 8. S.C., and 63 days afterwards was inoculated subcutaneously with 50 mg. of culture of B XXVI. The animal was killed 92 days after the second inoculation.

Lungs.

In two specimens, taken from different parts of the lungs, several consolidated foci have been found. In one of these there is a caseous patch which is commencing to calcify. In this caseous material numerous bacilli are present. The majority of the lesions are not caseous, but contain many giant cells and fibroblasts, together with a sprinkling of leucocytes; tubercle bacilli are present in most of them but are rare. These tubercles are not surrounded by a definite layer of fibrous tissue, but from their general characters, viz., tendency to fibroid change, frequency of giant cells, and rarity of bacilli, they appear to be retrogressive.

Liver.

In the two different specimens which have been examined no lesions or bacilli have been found.

Kidneys.

No lesions have been found, but a small group of bacilli is present within one glomerulus.

Spleen.

No lesions or bacilli have been found.

Bronchial Gland.

There are several small patches which are slightly caseous and contain many giant cells. Bacilli are present in these areas but are scanty.

SUMMARY.

In the lungs several retrogressive lesions are present; in one caseous focus, however, bacilli are numerous. There is very little evidence of dissemination elsewhere.

(7.)

VIRUSES—{H 8. S.C.
B XXVI.

Animal Inoculated.—Calf 1025.

Calf 1025 was first inoculated subcutaneously with 50 mg. of culture of H 8. S.C., and 63 days afterwards was inoculated subcutaneously with 50 mg. of culture of B XXVI. The animal was killed 91 days after the second inoculation.

Lungs.

Two specimens have been examined and in both of them caseous lesions are present. Surrounding the completely necrotic foci there are broad areas of consolidated tissue which are characterised by the presence of (1) numerous giant cells, (2) many newly formed capillaries containing red corpuscles, (3) an abundant infiltration of plasma cells, and (4) aggregations of oxyphil leucocytes. At the periphery of the tubercles there is a zone of fibroblasts.

Tubercle bacilli are present but are very rare.

Liver, Kidneys, Spleen.

In the liver (two specimens), kidneys (two specimens), and spleen (one specimen) neither tubercles nor tubercle bacilli have been found.

Bronchial Gland.

Ramifying, caseous tubercles are present, and there are numerous giant cells. Bacilli are rare.

SUMMARY.

Fairly extensive caseous tubercles, of the chronic type and containing very few bacilli, are present in the lungs and bronchial glands. No lesions have been found elsewhere.

(8.)

VIRUSES—{H 8. S.C.
B XXVI.

Animal Inoculated.—Calf 1027.

Calf 1027 was first inoculated intravenously with 10 mg. of culture of H 8. S.C. and 63 days afterwards was inoculated subcutaneously with 50 mg. of culture of B XXVI. The animal died 46 days after the second inoculation.

Lungs.

In general appearance the lungs are in the condition which is usually produced by subcutaneous inoculation of a large dose of a virulent virus. There are large ramifying caseous areas, and there is an abundant fibrinous deposit; no giant cells are present, and there is no evidence that the development of the lesions is being arrested. Plasma cells are very abundant at the periphery of some of the caseous tracts, perhaps more abundant than is usual in acute infections. Tubercle bacilli are numerous and vary in length from about 2 to 4 μ. Many of them are curved. Beading is not common.

Liver.

Small, necrotic tubercles are present but are not numerous. They are generally without any definite boundary zone, and some of them contain a fibrinous deposit. Bacilli are scanty.

Kidneys.

A few caseous tubercles are present. They contain no fibrin. Bacilli are moderately numerous.

Popliteal Gland.

A few small, caseous foci are present. Bacilli are not numerous.

Summary.

Disseminated tuberculosis, with severe incidence on the lungs. In other organs there is less evidence of acute disease than is usual with non-vaccinated calves after subcutaneous inoculation of 50 mg. of virulent culture.

(9.)

$$\text{VIRUSES—}\left\{\begin{array}{l}\text{H 8. S.C.}\\ \text{H 56. F.T.}\\ \text{B XXVI.}\end{array}\right.$$

Animal Inoculated.—Calf 1033.

Calf 1033 received the following inoculations:—(1) 50 mg., subcutaneously, of culture of H 8. S.C.; (2) 55 days afterwards, 50 mg., intravenously, of culture of H 56. F.T.; (3) 114 days after the first inoculation, 50 mg., subcutaneously, of culture of B XXVI. The animal was killed when in good health 90 days after the last inoculation.

Lungs.

In the specimen examined there is a large tubercle which contains numerous giant cells and is slightly caseous. It is abundantly infiltrated with lymphocytes, both small and large. Bacilli are scanty.

Liver, Kidneys, and Spleen.

No tubercles or tubercle bacilli have been found in these organs, but there is some interstitial infiltration in the kidneys.

Prescapular Gland.

In the specimen examined there are a few minute lesions containing a few bacilli.

Summary.

A few non-progressive tubercles are present in the lungs, but there is no evidence of dissemination in other organs.

(10.)

$$\text{VIRUSES—}\left\{\begin{array}{l}\text{H 8. S.C.}\\ \text{H 56. F.T.}\\ \text{B XXVI.}\end{array}\right.$$

Animal Inoculated.—Calf 1035.

Calf 1035 received the following inoculations:—(1) subcutaneously, 50 mg. of culture of H 8. S.C.; (2) intravenously, 55 days afterwards, 50 mg. of culture of H 56. F.T.; (3) subcutaneously, 114 days after the first inoculation, 50 mg. of culture of B XXVI. The animal was killed when in good health 91 days after the last inoculation.

Lungs.

In the specimen examined there is a large consolidated patch which is partially caseous and contains some giant cells. Bacilli are scanty, but the consolidated area has no definite boundary zone and appears to be progressive. There is some evidence of fibrin.

Liver, Kidneys, and Spleen.

In the specimens examined neither lesions nor bacilli have been found.

Bronchial Gland.

A few caseous patches are present, but no bacilli have been found in them.

Summary.

The lungs contain consolidated areas of considerable size, which do not appear to be retrogressive. No lesions or bacilli have been found in the specimens of liver, kidneys, and spleen.

(11.)

$$\text{VIRUSES—}\left\{\begin{array}{l}\text{H 8. S.C.}\\ \text{B XXVI.}\end{array}\right.$$

Animal Inoculated.—Calf 1039.

Calf 1039 was inoculated (1) intravenously, with 10 mg. of culture of H 8. S.C., (2) subcutaneously, 63 days later, with 1 mg. of culture of B XXVI, (3) subcutaneously, 114 days after the first inoculation, with 50 mg. of culture of B XXVI. The animal was killed when in good health 93 days after the last inoculation.

Lungs.

Specimens from two different parts of the lungs show small, caseo-calcareous tubercles which are completely avascular and have a well-marked peripheral zone in which fibroblasts are plentiful. Some giant cells are present. Tubercle bacilli are fairly abundant in the caseous areas of the tubercles.

Liver, Kidneys, and Spleen.

In the specimens examined no lesions or bacilli have been found.

Prescapular Gland.

There are several caseo-calcareous tubercles, and giant cells are plentiful. The tubercles have generally a well-marked peripheral zone. Bacilli are fairly numerous.

SUMMARY.

In the lungs there are some caseous tubercles of an evidently chronic type but still containing bacilli in fairly large numbers. No evidence of dissemination has been found in the specimens of liver, kidney, or spleen.

(12.)

VIRUSES—{H 8. S.C. / B XXVI.

Animal Inoculated.—Calf 1043.

Calf 1043 received the following inoculations. (1) 10 mg., intravenously, of culture of H 8. S.C.; (2) 63 days afterwards, 1 mg., subcutaneously, of culture of B XXVI; (3) 51 days after the second inoculation, 50 mg., subcutaneously, of culture of B XXVI. The animal died, apparently from diarrhœa, 53 days after the last inoculation.

Lungs.

Two specimens have been examined. In one there is some congestion and general thickening of the alveolar walls, but no tubercles or tubercle bacilli. In the other there is an area of incomplete consolidation which contains a few caseous patches. Throughout this area tubercle bacilli are scattered, and some rather large groups are present. There is an infiltration of leucocytes and lymphocytes but very little evidence of fibrous tissue formation. There is no indication that this lesion is in a retrogressive condition.

Liver, Kidneys.

In two specimens of each of these organs neither tubercles nor tubercle bacilli have been found.

Bronchial Gland.

A few small caseous patches are present, containing giant cells and small numbers of bacilli.

SUMMARY.

There is some infection of the lungs, which is apparently not quiescent, but very little sign of dissemination elsewhere.

(*b*) CONTROL EXPERIMENTS WITH B XXVI.

The virus B XXVI proved to be of high virulence when inoculated subcutaneously into two very young calves (age about 4 weeks) with a dose of 50 mg. Both these animals died from generalised tuberculosis, one in 76 days and the other in 44 days. Two older animals were also used as controls, their age being about the same as that of the vaccinated animals at the time when their power of resistance was tested. I have made the following observations on the histology of the lesions produced in these two latter animals:—

(1.)

Animal Inoculated.—Calf 474.

Calf 474, when 17 weeks old, was inoculated subcutaneously with 50 mg. of culture of B XXVI, and was killed in good health 92 days afterwards.

Lungs.

There are numerous consolidated areas, irregular in outline and in distribution, which are partially caseous and contain a few giant cells. Plasma cells, many of them passing into fibroblasts, form a well marked layer at the periphery of these areas and are also numerous in their interior. Tubercle bacilli are rather scanty. There are some remains of a fibrinous deposit. Owing to their extent, irregularity of outline, and the large amount of caseous deposit, the lesions appear to be progressive; but the relative scarcity of bacilli and the evident attempts at the formation of a demarcation zone indicate that they are only slowly progressive.

Liver, Kidneys, and Spleen.

In the specimens examined no tubercles or tubercle bacilli have been found.

Bronchial Gland.

The gland is extensively caseous and contains numerous giant cells. The lesions are evidently progressive, though there is an imperfect layer of fibroblasts at the periphery of some of them. Bacilli are moderately numerous.

SUMMARY.

Compared with the usual result produced by 50 mg. of a virulent virus, the amount of disease is slight; the lungs are in a condition of slowly progressive tuberculosis.

(2.)

Animal Inoculated.—Calf 476.

Calf 476 was inoculated subcutaneously, when 19 weeks old, with 50 mg. of culture of B XXVI, and died 57 days afterwards.

Lungs.

The tissue is nearly completely solidified, contains irregular, ramifying, caseous, and necrotic areas, and shows no evidence of a demarcation zone at the periphery of the tubercles. Bacilli are abundant.

Liver.

Small, miliary tubercles are scattered throughout the tissue. They have no cellular boundary zone, are devoid of giant cells, and contain moderately numerous bacilli.

Spleen.

The tissue is extensively caseous; the lesions show no evidence of limitation by a boundary zone. Bacilli are fairly numerous.

Bronchial Gland.

The tissue is almost completely replaced by caseous material and contains large numbers of bacilli.

SUMMARY.

The lesions are typical of acute, fatal infection.

(c) ANALYSIS AND COMPARISON OF RESULTS.

When the virus B XXVI was compared with other viruses of bovine origin, by testing its virulence on young calves in a subcutaneous dose of 50 mg. of culture, fatal tuberculosis was produced in both the calves used for the experiment. One of them died in 76 days, the other in 44 days. The virulence of this virus may therefore be regarded as typical of the bovine bacillus.

The present series of observations provides a further illustration of the fact, to which attention was drawn in my previous Report (App. to 2nd Int. Rep., Vol. IV.), that though this particular test, the subcutaneous inoculation into very young calves of a dose not less than 50 mg., gives uniform results, under other experimental conditions the results are much less uniform. This feature is exhibited in the following parallel series :—

(1) Virulence highly irregular.

(*a*)

DOSE.—50 mg., subcutaneously, of B XXVI.

AGE OF CALVES.—Above the average.

Calf 474.	Calf 476.
Age.—17 weeks. Killed, "when well," 92 days after inoculation. The lungs are in a condition of slowly progressive tuberculosis. Elsewhere the amount of disease is slight.	Age.—19 weeks. Died 57 days after inoculation. The lesions in all the tissues examined are typical of acute fatal infection.

(*b*)

DOSES.—(1) 10 mg., subcutaneously, of H 54 C.W. (slightly virulent).
181 days afterwards
(2) 50 mg., subcutaneously, of H 8 S.C. (slightly virulent).
63 days afterwards
(3) 50 mg., subcutaneously, of B XXVI.

AGE OF CALVES.—Above the average.

Calf 923.	Calf 925.
Age.—13 weeks. Died 36 days after the last inoculation. Lungs severely affected. Relatively little disease in the other tissues examined.	Age.—13 weeks. Killed, in good health, 90 days after the last inoculation. No evidence of disease in any of the tissues examined.

(*c*)

DOSES.—(1) 10 mg., intravenously, of H 8. S.C. (slightly virulent).
63 days afterwards
(2) 50 mg., subcutaneously, of B XXVI.

AGE OF CALVES.—About the average.

Calf 1017.	Calf 1027.
Age.—6 weeks. Killed, in good health, 95 days after the second inoculation. The tissues examined are almost entirely free from disease.	Age.—6 weeks. Died 46 days after the second inoculation. Lungs acutely affected. Lesions of moderate severity in other organs.

(2) Virulence less irregular or uniform.

(*a*)

DOSES.—(1) ·001 mg., subcutaneously, of B XXVI.
51 days afterwards
(2) 50 mg., subcutaneously, of B XXVI.

AGE OF CALVES.—About the average.

Calf 414.	Calf 416.
Age.—5 weeks. Died (not from tuberculosis), 93 days after the second inoculation. The tissues examined show no evidence of dissemination beyond the nearest glands.	Age.—5 weeks. Killed, in good health, 89 days after the second inoculation. Tubercles, not actively progressive, are present in the lungs. Very little evidence of disease elsewhere.

(*b*)

DOSES.—(1) 50 mg., subcutaneously, of H 8 S.C. (slightly virulent).
63 days afterwards
(2) 50 mg., subcutaneously, of B XXVI.

AGE OF CALVES.—About the average.

Calf 1023.	Calf 1025.
Age.—5 weeks. Killed, in good health, 92 days after the second inoculation. Several lesions, probably retrogressive, in the lungs. Very little evidence of disease elsewhere.	Age.—8 weeks. Killed, in good health, 91 days after the second inoculation. Chronic caseous tubercles in the lungs. Very little evidence of disease elsewhere.

(*c*)

DOSES.—
(1) 50 mg., subcutaneously, of H 8 S.C. (slightly virulent).
55 days afterwards
(2) 50 mg., intravenously, of H 56 F.T. (slightly virulent).
59 days afterwards
(3) 50 mg., subcutaneously, of B XXVI.

AGE OF CALVES.—Above the average.

Calf 1033.
Age.—8 weeks.

Killed, in good health, 90 days after the last inoculation.

A few non-progressive tubercles in the lungs. No evidence of dissemination in the other tissues examined.

Calf 1035.
Age.—12 weeks.

Killed, in good health, 91 days after the last inoculation.

The lungs contain lesions which are of considerable size, and are not definitely retrogressive. Very little evidence of disease elsewhere.

(*d*)

DOSES.—
(1) 10 mg., intravenously, of H 8 S.C. (slightly virulent).
63 days afterwards
(2) 1 mg., subcutaneously, of B XXVI.
51 days afterwards
(3) 50 mg., subcutaneously, of B XXVI.

AGE OF CALVES.—Above the average.

Calf 1039.
Age.—9 weeks.

Killed, in good health, 93 days after the last inoculation.

The lungs show tubercles which are of a chronic type, but contain fairly numerous bacilli. Very little evidence of disease elsewhere.

Calf 1043.
Age.—11 weeks.

Died, apparently from diarrhœa, 53 days after the last inoculation.

The lungs contain some lesions which are not definitely retrogressive. Very little evidence of disease elsewhere.

(*d*) SUMMARY.

My histological observations support the following conclusions :—

(*a*) *Very young Calves.*

(1) A dose of 50 mg. of B XXVI, inoculated subcutaneously, produces fatal tuberculosis in normal animals.

(2) The subcutaneous inoculation, 51 days previously, of ·001 mg. of B XXVI greatly reduced the susceptibility of two calves, each 5 weeks old, to a dose of 50 mg. of the same virus.

(3) An equally marked reduction in susceptibility was produced in two calves, one 5 and the other 8 weeks old, by the previous inoculation, subcutaneously, of 50 mg. of a human virus possessing low virulence for the bovine.

(4) When the same slightly virulent virus was introduced intravenously, in a dose of 10 mg., into two calves, each 6 weeks old, the results were unequal. One animal proved highly resistant to the subsequent test inoculation ; the other succumbed to fatal infection.

(*b*) *Older Calves.*

(1) A dose of 50 mg. of B XXVI, inoculated subcutaneously into two normal calves, produced unequal results, the infection being mild in one animal (17 weeks old) and rapidly fatal in the other (19 weeks old).

(2) Previous treatment with two doses of slightly virulent human viruses (50 mg. subcutaneously, followed by 50 mg. intravenously) rendered two calves (8 and 12 weeks old) much less susceptible to inoculation with 50 mg. of B XXVI ; but the increased resistance was not more marked than in the case of the two younger calves (5 and 8 weeks old) which received only one dose of slightly virulent bacilli (50 mg. subcutaneously).

(3) Previous treatment with 10 mg. of slightly virulent human bacilli (intravenously), followed by a second vaccination with 1 mg. of B XXVI (subcutaneously), increased the resistance of two calves (9 and 11 weeks old) ; but the increased resistance was not more marked than in the case of the two younger calves (each 5 weeks old) which received only one vaccination (·001 mg. of B XXVI subcutaneously).

(4) Previous treatment with two protective subcutaneous doses (10 mg. followed by 50 mg.) of slightly virulent human bacilli produced unequal results in two calves, each aged 13 weeks. One showed very high resistance. The other was fatally infected.

II.—ADDITIONAL OBSERVATIONS ON BOVINES.

(1) *Early Dissemination.*

VIRUSES—
B XXIX.
B XXX.

Animal Inoculated.—Calf 438.

Calf 438 was inoculated subcutaneously with 44 mg. of culture of B XXIX and B XXX, and was killed twenty-four hours afterwards.

In two different portions of each of the following organs neither lesions nor bacilli have been found :— lungs, liver, kidneys, and spleen. The examination of a bronchial, a portal, and a popliteal gland also gave negative results.

Local Lesion.

Bacilli are abundant, and there is a dense infiltration with multinuclear leucocytes.

Prescapular Gland.

Bacilli are very rare.

SUMMARY.

There is no evidence, in histological sections, of dissemination beyond the nearest gland. Drs. A. S. and F. GRIFFITH have, however, by culture and inoculation tests, demonstrated the presence of bacilli in the blood, lungs, spleen, liver, kidneys, marrow, muscle, and a popliteal gland.

(2) *Cutaneous Inoculation.*

VIRUS—B XXVI.

Animal Inoculated.—Calf 466.

Calf 466 received a cutaneous inoculation of 50 mg. of culture of B XXVI, and was killed 139 days afterwards.*

Lungs.

There are a few slightly caseous foci which contain several giant cells. One tubercle bacillus has been found.

Liver, Kidneys, and Spleen.

No tubercles or bacilli have been found.

Gluteal Gland.

The gland is extensively caseous and contains calcareous foci. The lesions are obviously retrogressive in character. One or two tubercle bacilli have been found.

Skin.

The skin at the site of inoculation shows chronic caseous tubercles with numerous giant cells. Bacilli are very scanty.

SUMMARY.

It appears probable that very few bacilli have been absorbed through the skin.

(3) *Cows B, C, and D.*

These three cows were affected with spontaneous tuberculosis, but their udders appeared normal, both during life and at the post-mortem examination. Tubercle bacilli were, however, shown by guinea-pig inoculations to be present in the milk yielded by the first two cows.

Histological specimens have been prepared from each of the four quarters of each udder. In none of the specimens have either tubercle bacilli or histological tubercles been found.

The specimen of lung from Cow D shows caseous tubercles in which bacilli are rare.

III.—LESIONS IN RABBITS.

(*a*) Early Stages of the Infective Process after Subcutaneous Inoculation.

Dr. F. GRIFFITH inoculated a series of rabbits subcutaneously, over the scapula, each with a dose of 50 mg. of bovine culture. One animal was killed at each of the following intervals after inoculation:—24 hours, 3 days, 6 days, 8 days, and 14 days.

Twenty-four Hours.

At the site of inoculation (Rabbit 1542) there is a dense mass of bacilli. At the margins of this mass bacilli are more loosely scattered, and a few have been found amongst the fibres of the subjacent muscle, but the amount of dissemination is only slight, and the great bulk of the inoculated material remains as a compact lump of culture. There is no sign of vaso-dilation; the number of blood-capillaries is scanty, and no bacilli have been found in any of those present. The culture mass is crowded with cells which are almost entirely leucocytes; many of these cells have lost their granules, and loose granules are found scattered about. Some of the cells are filled with bacilli. In addition to the ordinary multinuclear form, mononuclear oxyphil leucocytes are frequent. The nuclei of some of the cells stain faintly, and others are broken into fragments. Passing from the central mass of bacilli to the region where bacilli are loosely scattered, leucocytes are very abundant, and the great majority of them are normal in appearance; a few of them contain bacilli; loose bacilli are also found. Completely outside the zone of bacilli leucocytes are still very plentiful; these cells seem to be converging towards the bacilli from all directions. Some of the bacilli are distinctly longer than serum grown bacilli, and a few are beaded or irregularly stained.

Specimens have been searched of lung, liver, kidney, and spleen, but no bacilli have been found.

Three Days.

The centre of the inoculated material (Rabbit 1543) consists of a dense mass of bacilli. External to this mass bacilli are loosely scattered in greater numbers than in the former specimen, but not many have penetrated into the muscular tissue. Blood-vessels are scanty and not dilated; no bacilli have been found in them. Leucocytes are abundant in the mass of culture, but show more complete loss of granulation and more disruption of their nuclei than in the former specimen. Throughout the areas of loosely scattered bacilli leucocytes are very abundant; they are more frequently phagocytic than in the former specimen; the majority of them have not retained more than a few granules in their protoplasm, and many, though their protoplasmic outline is intact, show no granules at all; in many cases their nuclei stain faintly. Leucocytes are also abundant, but generally normal in appearance, in the situations where bacilli are scanty and also in the adjacent tissue where no bacilli have arrived. Many of the loose bacilli are longer than serum grown bacilli and show a tendency to beading.

No bacilli have been found in the specimens examined of lung, liver, kidney, and spleen.

Six Days.

In the local lesion (Rabbit 1544) bacilli are still very abundant, but are more loosely distributed than in former specimens. In the central areas leucocytes are still abundant, but a much larger percentage of them are broken down; there is also a further accession of fresh leucocytes, as evidenced partly by the free distribution of granules shed from these cells and partly by the presence of intact granular leucocytes; many of the intact leucocytes in these central situations contain bacilli. There is also a tendency for small lymphocytes to penetrate into the centre of the lesion. Passing outwards, the bacilli are loosely scattered and rapidly decrease in number; there is also a marked change in the cellular infiltration, the leucocytes rapidly diminishing and the lymphocytes greatly increasing. At a short distance from the central focus the infiltration of small lymphocytes, together with an admixture of large lymphocytes and plasma cells, forms a wide and compact zone. The blood-capillaries are rather more numerous than in former specimens; they are dilated, exhibit marked swelling of the endothelial cells, and, very occasionally, contain bacilli.

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* Another animal, Calf 452, was cutaneously inoculated with 10 mg. of the same virus, and was killed 129 days afterwards. The specimens examined of skin, a gluteal gland, and a precrural gland show neither lesions nor bacilli.

In the lungs there are a few bacilli, which are situated in small, slightly caseous, lesions containing many leucocytes. No bacilli have been found in the specimens of liver, kidney, or spleen.

Eight Days.

The central part of the local lesion (Rabbit 1545) has liquefied. In the adjacent solid portion, bacilli, though numerous, do not occur in dense masses; the leucocytes are still actively phagocytic, but there is a greater proportion of lymphocytes. The outer portions of the lesion are in the same condition as in the last specimen, but there is an increase in the number of cells which give a well marked reaction to Pappenheim's stain.

No bacilli have been found in the specimens examined of lung, liver, kidney, and spleen.

Fourteen Days.

The general condition of the local lesion (Rabbit 1546) is the same as in the last specimen, but there is a further diminution in the numbers of bacilli and also of the leucocytes. The lymphocytes and endothelial cells show no further change, but the number of plasma cells in the peripheral areas has increased.

In the specimen of lung a few suspicious foci have been found, but no bacilli. In the liver two bacilli have been found, and there are a few small lesions. In the kidney no lesions or bacilli have been seen. In the spleen there are a few minute lesions of doubtful nature, but no bacilli have been found.

SUMMARY.

Notwithstanding the large doses of highly virulent bacilli, there is remarkably little evidence of disseminated lesions during the first fortnight after inoculation.

(*b*) Lesions Produced by Dead Bacilli.

(1.)

VIRUS—B XXVI.

Animal Inoculated.—Rabbit 488.

Rabbit 488 was inoculated, intravenously and intraperitoneally, with dead bacilli from cultures of B XXVI (the total dose given amounting to 140 mg.), and was killed 63 days afterwards.

Lungs.

The lungs contain numerous deposits of bacilli. Some of these consist of clumps of bacilli which even in their smallest diameter are many times the thickness of a blood capillary; the presence of these deposits is difficult to explain except by supposing that they are due to successive deposits of bacilli carried by the blood-stream and accumulated round an initial obstruction. In other places the bacilli, though forming a large deposit, are not solid masses but are more or less separate from each other; it is evident that these deposits have been derived from the blood-stream by a process of gradual accumulation. Bacilli also occur in many places either (1) as isolated, minute, solid clumps which could easily have travelled along the capillaries, or (2) as groups of individual bacilli. With the exception that many of them are broken up, the bacilli resemble serum grown bacilli.

The deposits of bacilli have given rise to well marked and characteristic tubercles. At the periphery of these tubercles there is a well marked layer of plasma cells, together with transitional forms between plasma cells and fibroblasts, and fibroblasts which give no pyronin reaction. Internal to this layer there are epithelioid cells and many giant cells. Oxyphil leucocytes are numerous at the periphery of the tubercles and in the interior form dense masses aggregated round the bacilli. No fibrin is present.

Liver.

In the specimen examined there is a caseous tubercle with a broad cellular periphery. In the peripheral area plasma cells are very numerous; oxyphil leucocytes are less abundant. There are many newly formed capillary channels containing a few red corpuscles. A few giant cells are present. No tubercle bacilli have been found.

Kidneys.

There is no formation of tubercles, but a few tubercle bacilli have been found within blood-vessels.

SUMMARY.

Typical tubercles of the chronic type are present in the lungs and liver. In the lungs there are numerous deposits of dead bacilli; these are being vigorously attacked by the oxyphil leucocytes.

(2.)

VIRUS—Bovine.

Animal Inoculated.—Rabbit 1283.

Rabbit 1283 was inoculated intravenously with 50 mg. of dead bacilli, obtained from cultures of various bovine viruses, and was killed 29 days afterwards.

Lungs.

Bacilli are present in most parts of the tissue but are not numerous; with the exception of a few very small clumps they occur either singly or in groups of half a dozen or less. No bacilli have been found within blood-vessels.

Throughout the tissue the lung alveoli are very greatly thickened, and many patches are completely solidified. The places where bacilli are demonstrable are generally the centres of small areas of slight caseation.

The foci are characteristic of the more chronic type of lesion set up by living tubercle bacilli ; they contain giant cells, numerous epithelioid cells, and a peripheral zone of fibroblasts and lymphocytes. In the lesions where bacilli are very scanty and occur singly, oxyphil leucocytes are not very numerous and are not in close contact with the bacilli ; but the small clumps of bacilli which have survived are surrounded with clusters of oxyphils.

Liver.

Single bacilli and fragments of bacilli have been found in many places, but bacilli are much less numerous than in the lungs ; none have been found within blood-vessels.

The most striking feature is the presence of numerous giant cells in all parts of the section. These cells occur singly, not as portions of larger lesions, and are surrounded by normal tissue. In several of them a tubercle bacillus is present. Most of the giant cells are in a very early stage of formation. Their protoplasm arises from a fusion of the protoplasm of a group of parenchymatous liver cells, and their nuclei can sometimes be identified with the nuclei of these cells. Associated with their formation there is a swelling of the capillary endothelium, and the nuclei of these latter cells generally contribute to the nuclei of the giant cell, sometimes obliterating or replacing the nuclei of the parenchymatous liver cells. The nuclei of these endothelial cells divide amitotically ; in one situation this process is seen in a cell partially detached from the vessel wall and at the periphery of a giant cell.

Kidneys.

No lesions or bacilli are present.

Spleen.

There are no tubercles or giant cells, but in a few places small numbers of bacilli, often fragmentary, are present. These are generally associated with small groups of oxyphil leucocytes. The nuclei of many of the leucocytes are broken up.

SUMMARY.

The lungs contain small numbers of bacilli and show many small lesions which are characteristic of chronic tuberculosis ; probably much of the consolidation is due to the irritant effects consequent on inoculating so large a dose. The presence of bacilli in the liver has led to the production of numerous giant cells.

(3.)

VIRUS—Bovine.

Animal Inoculated.—Rabbit 1284.

Rabbit 1284 was inoculated intravenously with 10 mg. of dead bacilli, obtained from cultures of various bovine viruses, and was killed 29 days afterwards.

Lungs.

Bacilli occur in many parts of the tissue and have led to the production of small caseous foci similar to those found in the lungs of Rabbit 1283. In many of these foci bacilli are rare ; in some a small clump is present. The bacilli are not surrounded with leucocytes. Apart from these foci there is much less tendency to consolidation than in the lungs of Rabbit 1283. No bacilli have been found within blood-vessels.

Liver.

Under a low power the tissue appears normal. With a higher magnification a few tubercle bacilli can be found. They are always closely associated with the portal veins and the venous capillaries. The bacilli have produced very little tissue reaction. Sometimes they are simply contained within endothelial cells, without causing any change in the surrounding tissue, and occasionally there is a group of bacilli surrounded by endothelial cells and small lymphocytes.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Identical with the spleen of Rabbit 1283.

SUMMARY.

The lungs contain tubercles identical with those of the rabbit inoculated with the larger dose, but there is less evidence of irritant action in the surrounding tissue. In the liver there are no giant cells, and the bacilli have not succeeded in escaping beyond the capillary walls.

General Results of Inoculation with Dead Bovine Bacilli.

When dead tubercle bacilli are carried to the internal organs by the blood-stream the nature of the lesions produced depends largely upon the quantity of bacilli injected and the fineness of the emulsion. If, owing to the magnitude of the dose and the presence of many small clumps, foci are formed which contain large deposits of bacilli, the lesions bear a close resemblance to chronic tubercles produced by living bacilli. They have a peripheral zone of fibroblasts, plasma cells and transitional forms between plasma cells and fibroblasts ; internal to this there is a zone of epithelioid cells and giant cells ; the central area is necrotic ; and passing inwards from the periphery to the centre there is an abundant sprinkling of leucocytes. But the presence of a large, centrally placed mass of bacilli is a feature which often distinguishes these lesions from those produced by living bacilli. An equal number of living bacilli would have been much more loosely distributed and would have caused more wide-spread tissue damage ; whilst in the ordinary chronic tubercle, such as these lesions resemble, bacilli are usually rare or absent. This difference is well illustrated in the lungs of Rabbit 488. A special feature about this tissue is the large size of some of the accumulations of bacilli, indicating that they must have been deposited at successive intervals. It is natural to suppose that a site already damaged by the presence of bacilli would effectively detain all fresh arrivals by the blood-stream, and thus would expand by a process of passive accumulation. Similarly with living bacilli, tubercles already formed would detain any circulating bacilli more readily than normal tissue; hence their expansion may be assisted by the accession of fresh arrivals and may not always be entirely due to the multiplication of the bacilli initially deposited.

Another feature of the tubercles containing a central mass of dead bacilli is the presence, not merely within the lesions but in actual contact with the bacilli, of large masses of oxyphil leucocytes possessing a well defined granulation. Had the bacilli been alive, the leucocytes in proximity to them would have been disintegrated. But though the toxicity of the dead bacilli for the leucocytes is relatively feeble, it seems clear, from a comparison of different lesions, that the bacilli are in slow process of disappearance; and this disappearance seems to be associated with a breaking down of some of the leucocytes present, and may be due to the digestive ferments liberated by these cells. When the bacilli have all disappeared, or when they only survive in small numbers, the appearance of the lesions is quite indistinguishable from that of chronic tubercles produced by living bacilli.

When the emulsion of dead bacilli has been very finely divided, the lesions are usually smaller, though of the same type as before. Their size varies according to the number of bacilli deposited. The number of leucocytes present is less, and in the smallest of the chronic tubercles produced there may be few or none of these cells. There is also, as is well shown in the liver of Rabbit 1283, a remarkably large number of giant cells which occur singly, not as portions of larger lesions, and are surrounded by normal tissue. Many of them contain no more than one bacillus. Their protoplasm arises from a fusion of the protoplasm of a group of parenchymatous cells, and some of their nuclei can often be recognized as due to the amitotic division of the nuclei of these cells. Concurrently with this process of formation, there is a swelling of the capillary endothelium, and the nuclei of the endothelial cells generally contribute, by amitotic division, to the giant cell nuclei, sometimes obliterating or replacing the nuclei of the parenchymatous cells.

The intravenous inoculation of a large dose of dead culture, even though it be finely divided, produces, in addition to the lesions above noted, a general irritant effect, which is particularly well marked in the lungs. When the dose is diminished, this irritant effect is greatly reduced and it is also noticed that many of the bacilli, actually demonstrable in tissues such as the liver, are still entangled in the vascular and capillary endothelium and have not gained access to the parenchymatous cells. This circumstance may be attributable to a greater defensive power of the endothelial cells, and it may be suggested that one effect of the larger dose is to diminish the normal resistance of the endothelial cell. Perhaps the toxicity of large intravenous doses of feebly virulence living bacilli is partly due to the same action.

IV.—LESIONS IN FOWLS AND PARROTS.

(*a*) Fowls.

(1.)

VIRUS—B IV.

Animal Inoculated.—Fowl 414.

Fowl 414 was inoculated intravenously with 10 mg. of B IV, and died 21 days afterwards.

Lungs.

The lungs contain many minute foci which are slightly necrotic but are not characteristic of definite tubercles. In these foci bacilli are numerous. The bacilli are frequently beaded and some of them show signs of disintegration.

Liver.

Many minute foci are present; they are similar to those found in the lungs, but bacilli are less numerous.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Many small deposits occur and are similar in character to those found in the liver. The bacilli, though fairly numerous in some of the foci, show no definite signs of multiplication.

SUMMARY.

Small, disseminated deposits of bacilli, without evidence of active growth. No definite tubercle formation.

(2.)

VIRUS—B IV.

Animal Inoculated.—Fowl 420.

Fowl 420 was inoculated intravenously with 10 mg. of B IV, and died 20 days afterwards.

Lungs.

The lungs contain many large, slightly caseous, consolidated foci, at the margins of which there is a tendency to fibroid change. Bacilli are scattered throughout the lesions, and in a few places are fairly plentiful, though they exhibit no definite evidence of recent growth.

Liver.

There are many minute foci showing slight necrotic change ; bacilli are scanty.

Kidneys.

A few bacilli are deposited both within glomeruli and in the interstitial tissue, but there are no definite histological changes associated with them.

Spleen.

There are many minute foci similar to those found in the liver.

SUMMARY.

Similar to Fowl 414 but with more extensive tissue change in the lungs.

(3.)

VIRUS—B IX.

Animal Inoculated.—Fowl 492.

Fowl 492 was inoculated intravenously with 10 mg. of B IX, killed by steaming at 100° C., and was killed 35 days afterwards.

Lungs.

There are many fairly large patches of caseation with a peripheral area of fibroblasts. Some of the lesions present the characteristic appearances of definite tubercles of a chronic type. The lesions contain numerous bacilli. The tissue changes are similar, and equal in extent, to those in Fowl 420, which was inoculated with living bacilli.

Liver.

The liver contains numerous small lesions which are of a chronic type and show a marked tendency to fibroid change. Bacilli are very rare.

Kidneys.

There are several small foci, centrally caseous and surrounded with fibroblasts. Bacilli are very rare.

Spleen.

There are several minute patches of caseation. Bacilli are very rare.

SUMMARY.

The bacilli are generally disseminated, and have produced as much tissue change as is usually found in fowls inoculated with living mammalian bacilli.

(4.)

VIRUS—B XXVI.

Animal Inoculated.—Fowl 80.

Fowl 80 was inoculated intravenously with 1 mg. of culture of B XXVI, and was killed 102 days afterwards.

Lungs.

The lungs are studded with groups of tubercles which are circular in outline, completely necrotic in the centre, and generally surrounded by fibroblasts. There are no definite giant cells, but surrounding the necrotic areas there is a zone of cells of epithelioid type, and many of these cells are partially fused together. Oxyphil leucocytes are not numerous. At the periphery of the tubercles plasma cells are found and often form conspicuous groups. No fibrin is present. Tubercle bacilli are present in all the lesions and are numerous in most of them ; in several of the lesions the bacilli form groups visible with a low power. The bacilli are most numerous in the necrotic parts of the tubercles. They are loosely scattered and nowhere form solid lumps or clusters. They vary from about 2 to 4·5 μ in length and are often beaded.

Liver.

There are some irregular, partially necrotic areas in which masses of multinuclear leucocytes occur. In one of these lesions two tubercle bacilli are present. None have been found elsewhere.

Kidneys.

A small lesion of doubtful nature has been found, but no tubercle bacilli.

Spleen.

There are many small foci in which the cells stain somewhat faintly ; in some of these, fragments of bacilli and occasionally whole bacilli occur.

SUMMARY.

The lungs contain many caseous circumscribed tubercles in which bacilli are abundant ; from their morphology and distribution it is evident that the bacilli have multiplied after arriving in the lungs. There is evidence of dissemination in the liver and spleen, but in these organs bacilli are very rare.

(5.)

VIRUS—B XXVI.

Animal Inoculated.—Fowl 82.

Fowl 82 was inoculated intravenously with 10 mg. of culture of B XXVI, and died 53 days afterwards.

Lungs.

The lungs contain numerous foci which are circular in outline and are sharply circumscribed by fibroblasts. These foci vary in size, the largest being about the area of the microscopic field (low power) and the smallest only a few cells in diameter. Many of them are necrotic in the centre. They often occur in groups, smaller foci being present at the periphery of the larger. Oxyphil leucocytes, with a well marked granulation, are present in all the lesions. At the periphery they occur singly, but in the centres of many foci they form dense masses, and the areas of commencing necrosis are often filled with the nuclear fragments and the discharged protoplasmic granules of these cells. Cells which react well to Pappenheim's stain for plasma cells are numerous at the periphery of the lesions, but only occur scantily in the interior. The cellular groundwork of the lesions is formed by fibroblasts and cells, with a large amount of protoplasm and irregular outlines, which are probably of endothelial origin. Cells of this last type are often found partially fused together, but no definite giant cells have been met with. In all the lesions tubercle bacilli are present and in most of them they are abundant. They are generally loosely scattered, but in the centres of some of the lesions they are closely packed in groups. The bacilli are present at the periphery of the tubercles as well as in the centre, but are much more numerous in the latter situations and are obviously associated with the large aggregations of oxyphil leucocytes. No bacilli have been found within blood-vessels. The bacilli are from 1·5 to 3·5 or 4 μ in length and are generally uniformly stained, though some beaded forms are met with. The longer forms are generally curved. It is quite clear, owing to their disposition and their greater length compared with bovine serum-grown bacilli, that the bacilli have multiplied after inoculation. They have sometimes grown into small clumps but do not form rosettes, nor have they any special tendency to intracellular growth.

Liver.

Throughout the liver substance there are innumerable minute foci which consist of spherical groups of semi-necrotic cells. Most of these foci show no infiltration with either leucocytes or other cells, and no peripheral zone, but a few are surrounded with a scanty layer of fibroblasts. No plasma cells have been found. Most of the foci contain either fragments of tubercle bacilli or one or two intact bacilli. It is clear that these foci are due to the action of the bacilli, and that the bacilli have been unable to survive or multiply.

Kidneys.

In the centre of the specimen examined there is a small necrotic area which contains a few groups of tubercle bacilli and is surrounded by fibroblasts; the peripheral zone also contains a few oxyphil leucocytes and a few plasma cells. In parts of the tissue histologically unaltered, an occasional bacillus has been found adherent to, or within, the walls of a blood capillary.

Spleen.

As in the liver, numerous minute areas of partial necrosis, showing no definite structure or tissue reaction, are present. These areas often contain one or two intact bacilli, and, still more frequently, fragments of bacilli. It is evident that the bacilli are in process of destruction.

SUMMARY.

In the lungs bacilli are numerous and many lesions are present resembling tubercles of the chronic type but not containing giant cells. In the other organs the bacilli have been freely disseminated, though not many intact bacilli remain to be seen; they have produced minute necrotic foci without the characteristic structure of tubercles.

(6.)

VIRUS—B XXVI.

Animal Inoculated.—Fowl 338.

Fowl 338 was inoculated intravenously with 6 mg. of culture of B XXVI, and died 19 days afterwards.

Lungs.

There are numerous small lesions, many of which are slightly caseous in the centre. The lesions have not a definite outline or structure; except in the breaking down areas they contain large numbers of lymphocytes and some plasma cells. Leucocytes are scanty; they are present at the periphery of the lesions, but rarely occur in the central areas. Most of the lesions contain small groups of bacilli; these are situated in the breaking-down areas. Many of the bacilli are broken up; a few are much longer than the average serum-grown bacilli. Though the bacilli present are numerous, there is no clear evidence that there has been an active increase of the inoculated material.

Liver.

Throughout the specimen there are many small foci, in each of which a few bacilli are present. Bacilli are much less numerous than in the lungs; they do not form groups, and many of them are broken up. The lesions show rather less tissue destruction than those in the lungs; they contain many small lymphocytes and some plasma cells; leucocytes are rather scanty.

Kidneys.

There are a few minute deposits of bacilli, which have produced slight tissue damage.

Spleen.

There are many small, slightly caseous, lesions, each containing a few bacilli. Leucocytes are rare in the lesions.

SUMMARY.

Bacilli are present in all the organs examined, but do not appear to be multiplying; they have produced slight tissue damage, but the lesions can hardly be called definite tubercles.

(7.)

VIRUS—B XXVI.

Animal Inoculated.—Fowl 412.

Fowl 412 was inoculated intravenously with 10 mg. of B XXVI, and died 27 days afterwards.

Lungs.

There are large, confluent patches of consolidation which are partially caseous. The lesions are infiltrated with numerous leucocytes and have no definite boundary zone. Bacilli are scattered about the lesions in moderate numbers; no definite signs of multiplication have been found.

Liver.

There are many slightly caseous foci with no definite boundary zone. Bacilli are found in most of the lesions, from 6 to 12 in each, and some of them are much longer than serum-grown bacilli.

Kidneys.

Bacilli are very rare and are not associated with any definite lesions.

Spleen.

There are many foci of slight caseation, each of which contains a few bacilli.

SUMMARY.

The bacilli are generally disseminated and have produced numerous patches of caseation, but are not multiplying freely.

(8.)

VIRUS—B XXVI.

Animal Inoculated.—Fowl 458.

Fowl 458 was inoculated intravenously with 10 mg. of B XXVI, and died 11 days afterwards.

Lungs.

There are many confluent patches of early caseation. Bacilli are numerous.

Liver.

There are many small, apparently progressive patches of early caseation. Bacilli are fairly numerous.

Kidneys.

Bacilli are present but very rare, and have produced no definite lesions.

Spleen.

There are many small foci of early caseation in which bacilli are scanty.

SUMMARY.

General dissemination, associated with early caseous change. No indication that the bacilli are dying out.

(*b*) Parrots.

VIRUS—B XXVI.

(1.)

Animal Inoculated.—Parrot 6.

Parrot 6 received an intramuscular inoculation with 10 mg. of culture of B XXVI, and died 113 days afterwards.

Lungs.

The lungs contain circumscribed tubercles in an advanced stage of caseation. These lesions contain masses of leucocytes and the remains of these cells. No bacilli have been found.

Liver.

The liver contains many small lesions. In these, the central area consists of liver cells and endothelial cells, which have undergone protoplasmic fusion and multiplication of their nuclei, leading to giant cell formations; the peripheral area is mainly composed of a broad zone of leucocytes. No bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

SUMMARY

There are fairly large and extensively caseous tubercles in the lungs; the liver contains numerous smaller lesions. No bacilli have been found.

(2.)

VIRUS—B XXVI.

Animal Fed.—Parrot 8.

Parrot 8 was fed with 42 mg. of culture of B XXVI, and died 57 days afterwards.

Lungs.

The tissue is highly congested and shows large patches of complete consolidation which are commencing to break down, though not definitely caseous. There is a sprinkling of leucocytes throughout the tissue, but there are no large collections of these cells. Bacilli occur everywhere, and are extremely numerous in the consolidated areas.

Liver.

The liver contains many small lesions resembling those found in the liver of Parrot 6, with the exception that their periphery contains only small numbers of leucocytes. Bacilli are scanty.

Kidneys.

No lesions or bacilli have been found.

SUMMARY.

Extensive consolidation of the lungs, associated with large numbers of bacilli. In the liver, many small lesions containing few bacilli.

(3.)

VIRUS—B XI.

Animal Inoculated.—Parrot 14.

Parrot 14 was inoculated intravenously with ·1 mg. of culture of B XI, and died 17 days afterwards.

Lungs.

The tissue is almost completely solid, and though not caseous contains many patches of broken down leucocytes. Bacilli are very abundant in all parts of the specimen; they are notably long and curved and show a tendency to grow together in the form of streaks and tufts.

Liver.

Bacilli are freely disseminated in large numbers throughout the tissue and within the blood-capillaries. There is a general infiltration of lymphocytes and leucocytes, but no caseation or tubercle formation.

Kidneys.

There are no definite lesions. Tubercle bacilli are irregularly distributed and in some places are fairly numerous.

Spleen.

Bacilli are profusely distributed, but have produced very little tissue change.

SUMMARY.

General dissemination and abundant multiplication of bacilli; consolidation of the lungs; no tubercle formation.

(4.)

VIRUS—B XI.

Animal Fed.—Parrot 16.

Parrot 16 was fed with 50 mg. of culture of B XI, and died 83 days afterwards.

Lungs.

The tissue is completely solid, extensively caseous, and contains very large numbers of bacilli.

Liver.

There are numerous small, caseous tubercles, and the tissue as a whole is infiltrated with lymphocytes and leucocytes. In the tubercles, many of which contain giant cells, bacilli are present in fairly large numbers.

Kidneys.

There are many small, slightly caseous lesions, in which bacilli are fairly plentiful.

Spleen.

The spleen is permeated with caseous areas containing numerous bacilli. There is a tendency to giant cell formation.

SUMMARY.

General dissemination of bacilli, with the formation of progressive, caseous tubercles. The lungs are very severely affected.

General Results.

(1.) FOWLS.

The fowl does not appear to be susceptible to the bovine bacillus except by the intravenous method of experiment.* And when the bacilli are introduced intravenously, they evidently find themselves in an unfavourable soil. They may be entirely eliminated; a few of them may survive, mostly in a fragmentary condition; or they may survive in larger numbers; or finally, they may not only survive but show some signs of multiplication. But, at the most, the lesions which they produce are of a chronic character, and there is no clear indication that they would have extended, had the experiments been of longer duration.

The lungs show much more evidence of tissue change than the other organs. In this respect the bovine bacillus differs in its action from the avian, since the fowl's lung is much less susceptible to the avian bacillus even after intravenous inoculation, than other organs, notably the liver and spleen. It is noteworthy that in the lungs of Fowl 80, for example, bovine bacilli, introduced in the relatively small dose of 1 mg., have survived for over 100 days and appear to have multiplied. They have produced lesions which may be fairly described as tubercles. These foci are circular in outline, completely necrotic in the centre, and are surrounded by fibroblasts and plasma cells. Between the fibroid zone and the necrotic area there is a zone of cells of epithelioid type, many of which are partially fused together, though these fusions have not advanced to definite giant cell formation. Leucocytes occur in the lesions, but not in large numbers. The lungs of Fowl 82, 53 days after inoculation with 10 mg., show lesions which contain numerous bacilli and in most respects resemble the tubercles found in Fowl 80. One notable difference, however, is that in Fowl 82 there are numerous leucocytes in association with the lesions; these cells are particularly abundant in the centres of the lesions and are evidently attracted to the groups of bacilli. In the lungs of the fowls which died from 19 to 21 days after inoculation, the lesions are in an earlier stage and do not exhibit a definite structure or boundary zone. Many of the bacilli which they contain are broken up; a few are markedly longer than serum-grown bacilli, an appearance commonly met with in bacilli which retain vitality but find their environment unfavourable for active multiplication.

In the livers of all the fowls the presence of small foci showing partial necrosis is evidence of infection. In the 11 days' animal bacilli are fairly numerous in the lesions; in the 19, 20, and 21 days' livers most of the lesions contain a few bacilli; in the 53 days' case they are rarer, and in the fowl killed 102 days after inoculation they are rarer still. Probably the bovine bacillus is not capable of permanent survival in the fowl's liver.

The spleens are in the same condition as the livers, and in passing from the earlier to the later lesions a similar progressive diminution of bacilli is found.

The kidneys are only very slightly affected.

It is noteworthy that in Fowl 492, inoculated with dead bacilli, there is as much tissue change as in usually found in fowls inoculated with living mammalian bacilli.

(2.) PARROTS.

In Parrot 6, 113 days after intramuscular inoculation, no bacilli have been found, but the lungs contain large and extensively caseous tubercles, and the liver shows many smaller lesions in which giant cell formation is conspicuous. In both organs large numbers of leucocytes are associated with the lesions.

Parrot 8, 57 days after feeding with a large dose, shows in the lungs extensive patches of consolidation. These are commencing to break down and contain very large numbers of bacilli. Possibly some of the bacilli reached the lung by the respiratory tract. Bacilli are scanty in the liver, but this organ contains many small lesions which are similar in structure to those in the liver of Parrot 6.

Parrot 14 shows general dissemination and abundant increase of bacilli after intravenous inoculation with a small dose, and Parrot 16 shows disseminated, progressive tuberculosis after feeding.

Parrot 16, 83 days after feeding with a large dose, shows severe disease of the lungs and general dissemination of bacilli, with the formation of progressive, caseous tubercles.

It is noticeable that in the parrots, as in the fowls, the lungs are more extensively affected than other organs. The lungs of the parrots show much larger areas of disease than those of the fowls.

V.—LESIONS IN OTHER ANIMALS.

(a) A Baboon and a Chimpanzee.

(1.)

VIRUS—B IV.

Animal Inoculated.—Baboon 76.

Baboon 76 was inoculated subcutaneously with a very small dose of culture of B IV. (estimated by the method of dilution as equivalent to ·00001 mg.), and died 89 days afterwards.

Lungs.

Large, isolated, caseous tubercles are present. They contain giant cells at their periphery, but are not surrounded by fibroblasts. Throughout the tubercles bacilli are scattered in small numbers.

Liver.

There are several small tubercles in the section. These are only slightly caseous and generally contain giant cells; the peripheral zone contains leucocytes and lymphocytes, but is not well marked. Bacilli are scanty.

Kidneys.

No tubercles or bacilli have been found in the specimen examined.

Portal Gland.

Patches of commencing caseation are present and contain several bacilli.

SUMMARY.

Disseminated tuberculosis. The tubercles are discrete and do not contain many bacilli, but show no sign of retrogressive change.

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* Fowls have been tested by other methods of experiment but have not yielded useful material for microscopic investigation.

(2.)

VIRUS—B IV.

Animal Inoculated.—Chimpanzee 24.

Chimpanzee 24 was inoculated subcutaneously with an extremely small dose of culture of B IV (estimated by the method of dilution as equivalent to ·00001 mg.), and died 107 days afterwards.

Lungs.

The lungs are semi-solid and contain large caseous areas which have no demarcation zone and are typical of acute tubercular pneumonia. Bacilli are very abundant.

Liver.

In the specimen of liver there is a large, almost completely caseous nodule which is surrounded by fibroblasts; within this nodule bacilli are numerous. Smaller foci infiltrated with leucocytes and showing no fibrous periphery also occur; these show very few bacilli.

Kidneys.

Large, caseous, ramifying tubercles are present. Bacilli are less numerous than in the liver.

Spleen.

Large, discrete tubercles are present and are almost completely caseous. Bacilli are scanty.

Inguinal Gland.

A few suspicious foci are present, but no tubercle bacilli have been found.

SUMMARY.

Highly acute tuberculosis of the lungs, with very large numbers of bacilli; extensive, but less acute, tuberculosis of the liver, kidneys, and spleen.

(*b*) Dogs.

(1.)

VIRUS—B IV.

Animal Fed.—Dog 116.

Dog 116 was fed with 10 mg. of culture of B IV, and was killed 98 days afterwards.

Lungs.

There are a few slightly caseous and apparently retrogressive lesions in which bacilli are rare.

Liver.

A few slightly caseous, obviously retrogressive lesions. Bacilli very rare.

Kidneys.

No lesions or bacilli have been found

Mesenteric Gland.

The gland contains large, progressive patches of caseation. Bacilli are numerous but are confined to the caseous areas.

SUMMARY.

The bacilli are multiplying freely in the mesenteric gland; but in the lungs and liver evidence of dissemination though present is only slight.

(2.)

VIRUS—B IX.

Animal Inoculated.—Dog 130.

Dog 130 was inoculated subcutaneously with 48 mg. of culture of B IX, and died 85 days afterwards. It was then discovered that the inoculation had been partly intraperitoneal.

Lungs.

There is a general thickening of the alveolar walls, with many small patches of consolidation. The latter are not like typical tubercles. Unlike ordinary acute lesions, they have not a central area of caseation; and unlike lesions of a more chronic type, there is no arrangement of the cells into zones and areas resembling the structure of a tubercle. Both leucocytes and lymphocytes are numerous in the lesions. Bacilli are very abundant. In the consolidated foci they have grown into large masses and form colonies; they also occur, both singly and in groups, throughout the rest of the tissue. The small amount of tissue destruction, notwithstanding the presence of very large numbers of bacilli, is noteworthy.

Liver.

The liver contains enormous numbers of bacilli. As in the lungs, they are present everywhere, but have only produced a small amount of tissue destruction, and have not caused the formation of caseous areas or lesions with the typical structure of tubercles. Bacilli are present in some of the blood-vessels.

Kidneys.

Bacilli are irregularly distributed, and have not led to the formation of definite tubercles. Occasionally the bacilli occur in large groups, but on the whole they are much less numerous than in the lungs or liver.

Spleen.

The whole of the tissue is swarming with bacilli. The amount of tissue destruction is relatively small.

SUMMARY.

General dissemination and enormous multiplication of bacilli; amount of tissue destruction small; no formation of typical tubercles.

(3.)

VIRUS—B XII.

Animal Inoculated.—Dog 160.

Dog 160 (a pup) was inoculated intravenously with 1 mg. of culture of B XII, and died 52 days afterwards.

Lungs.

The tissue is highly congested and is in a semi-solid condition. The consolidated areas are distributed irregularly, as is usual in tubercular infections, but show very little sign of caseation. Bacilli are moderately numerous in the lesions.

Liver.

There is a generalised infiltration of bacilli but no tubercle formation. Bacilli are numerous and are evidently multiplying freely. In all situations where they are plentiful, the parenchymatous cells are disintegrated and leucocytes and lymphocytes are numerous.

Kidneys.

Bacilli occur in many parts of the tissue but only in small numbers. They have not produced definite lesions.

Spleen.

There is a large caseous area in which bacilli are numerous. The rest of the tissue, in the specimen examined, does not show definite lesions but contains bacilli which are scattered about everywhere in twos and threes.

Portal Gland.

Bacilli are distributed all over the section, but do not form large masses and have produced very little tissue destruction. There are no definitely caseous areas.

SUMMARY.

General dissemination of bacilli ; advanced consolidation of the lungs ; extensive disintegration of the parenchymatous cells of the liver ; an absence of typical tubercle formation.

(*c*) Two Pigs.

(1.)

VIRUS—B X.

Animal Inoculated.—Pig 138.

Pig 138 was inoculated subcutaneously with 1 mg. of culture of B X, and was killed 92 days afterwards.

Lungs.

In the lungs there are numerous discrete lesions which are large and necrotic, and exhibit caseo-fibrous change. No bacilli have been found in them.

Liver and Spleen.

These organs contain many caseous nodules surrounded by thick fibrous walls ; no bacilli have been found in the lesions.

Kidneys.

No lesions or bacilli have been found.

SUMMARY.

Disseminated, retrogressive lesions, containing no demonstrable bacilli, after subcutaneous inoculation with a small dose of a virulent virus.

(2.)

VIRUS—Cow B.

Animal Fed.—Pig 216.

Pig 216 was fed with the faeces of Cow B, a spontaneously infected animal, during 8 days, the total dose being about 4·78 kilogrammes ; and was killed 100 days after the commencement of the experiment.

Lungs.

The lungs contain small lesions which are retrogressive in type. Tubercle bacilli are present but rare.

Liver.

Large, necrotic, retrogressive lesions are present. Only one tubercle bacillus has been found.

Kidneys.

No tubercles or bacilli have been found.

Spleen.

The spleen shows chronic lesions with a tendency to fibroid change. Bacilli are very rare.

Mesenteric Gland.

The gland examined is almost completely necrotic. Tubercle bacilli are scanty, but are less rare than in the other organs.

SUMMARY.

Disseminated tuberculosis of a chronic type.

(*d*) Ponies.

(1.)

VIRUSES—B XII and B XI.

Animals Inoculated.—Ponies 6 and 12.

Pony 6 was inoculated subcutaneously with 50 mg. of culture of B XII, and was killed 132 days afterwards.

Specimens of the following organs have been examined without the discovery of either lesions or tubercle bacilli:—Lungs, liver, kidneys, spleen, and a bronchial gland.

Pony 12 was inoculated subcutaneously with 50 mg. of culture of B XI, and was killed 124 days afterwards.

Specimens of the following organs show neither lesions nor tubercle bacilli:—kidneys, spleen, and an axillary gland. In the lungs there are a few small lesions of doubtful nature and in the liver there are several; but no tubercle bacilli have been found, and it cannot be regarded as certain that the lesions were produced by these organisms. In the local lesion, which is caseous and shows conservative change, tubercle bacilli are moderately numerous.

SUMMARY.

The animals appear to have completely resisted infection.

(2.)

VIRUS—B XI.

Animal Inoculated.—Pony 30.

Pony 30 was inoculated intravenously with 10 mg. of culture of B XI, and died 20 days afterwards.

Lungs

The lungs are semi-solid and exhibit many caseous patches of irregular outline. There is also an abundant desquamation of alveolar epithelium. Giant cells are not uncommon, and there is a tendency to fibroid change at the margins of the caseous areas. Tubercle bacilli can be found in most of the lesions but are not numerous.

Liver.

There are many small foci which are not definitely caseous but show partial disintegration of the liver cells. Tubercle bacilli are present in these areas but are scanty.

Kidneys.

One bacillus has been found in a glomerulus and is surrounded by a few leucocytes. Another bacillus has been found in a small patch of interstitial infiltration.

Spleen.

There are several minute patches of commencing caseation. Bacilli are scanty.

Popliteal Gland.

Small caseous lesions and giant cells are present. Bacilli are scanty.

SUMMARY.

The lungs are severely affected, though showing evidence of conservative change. The other organs are much less extensively diseased. Bacilli are scanty.

Summary of Results.

Baboon 76 and Chimpanzee 24 illustrate the effects of a very minute dose of bovine bacilli subcutaneously inoculated. In both cases there was general dissemination, and in both animals the lungs were more extensively affected than other organs. The chimpanzee was much more severely infected than the baboon. The lungs of the chimpanzee show highly acute disease and contain very large numbers of bacilli; in the baboon's lungs the tubercles are discrete and do not contain many bacilli, though they show no sign of retrogression.

Dog 116.—Feeding with 10 mg. of culture has produced disseminated disease of a mild type.

Dogs 130 and 160.—In both these animals there is severe infection but an absence of typical tubercle formation. In the former, 85 days after a subcutaneous and intraperitoneal dose of 48 mg., there is a very large multiplication of bacilli in the various organs examined, but remarkably little tissue destruction; this condition may be compared with that sometimes found in calves after intravenous inoculation with bacilli of relatively low virulence, or with the condition produced in rats after intraperitoneal inoculation with a large dose. In the latter animal, 52 days after intravenous inoculation with a much smaller dose, 1 mg., the general condition is the same; but the bacilli, though numerous, are much less abundant than after the large subcutaneous and intraperitoneal dose. Both cases appear to be examples of a condition characterized by (1) a diminished bactericidal action of the blood upon the tubercle bacillus, and (2), concomitantly with this change, a diminution of the resisting power of the endothelial cell, together with an increased permeability of the vascular endothelial linings. The consequences of this increased permeability appear to be twofold; (a) the bacilli pass readily through the endothelial lining, and (b) the vitality and resistance of the tissue with which they come into contact are lowered by the invasion of injurious fluid constituents from the blood.

Pigs 138 and 216.—In the former animal the subcutaneous inoculation of a small dose (1 mg.) of bovine bacilli has produced disseminated lesions which have become definitely retrogressive at the end of three months. In Pig 216 disseminated tuberculosis of a chronic type has been produced by feeding with the faeces of a cow.

Ponies 6 and 12.—These cases illustrate the capacity of the horse to resist completely subcutaneous inoculation with 50 mg. of bovine bacilli.

Pony 30.—Fatal infection of the lungs after intravenous inoculation of 10 mg.

B.—ADDITIONAL OBSERVATIONS ON LESIONS PRODUCED BY HUMAN VIRUSES.

I.—LESIONS IN CALVES.

(*a*) Strains of High Virulence.

Specimens have been examined of tissues taken from the following calves, in which fatal disease was produced by the subcutaneous inoculation of culture.

Virus.	Dose.	Number of Calf Inoculated.	Duration of Experiment in Days.
H 13. A.D. (Calf 1237).	50 mg.	1327	27
H 49. T.C. (Calf 1263).	50 mg.	1347	52
H 49. T.C. (Original material).	48 mg.	1501	45
H 53. D.H. (b). (G.P. from original material).	50 mg.	1507	63
H. 53. D.H. (b). (G.P. from original material).	50 mg.	1545	53
H 60. W.B. (Bronchial Gland).	50 mg.	1099	28
H 60. W.B. (Bronchial Gland).	10 mg.	1107	58
H 60. W.B. (Bronchial Gland).	10 mg.	1109	32
H 60. W.B. (Bronchial Gland).	50 mg.	1425	46
H 69. F.K. (Meninges).	50 mg.	1267	56
H 77. R.L.	50 mg.	1349	19
H 88. E.L.	50 mg.	1271	45
H 89. W.I. (Lung).	50 mg.	1351	44
H 90. I.P. (Calf 1383).	50 mg.	1489	42
H 90. I.P. (Calf 1471).	45 mg.	1493	21
H 100. R.S. (Calf 1419).	50 mg.	1511	39
H 100. R.S. (Calf 1419).	50 mg.	1517	22
H 100. R.S. (G.P. from original material).	50 mg.	1523	73
H 100. R.S. (Calf 1409).	50 mg.	1543	34
H 105. G.S. (Calf 1449).	50 mg.	1521	78
H 105. G.S. (Calf 1405).	50 mg.	1527	59
H 107. H.H. (Calf 1453).	50 mg.	1519	49
H 108. H.R. (Calf 1539).	50 mg.	1509	29
H 108. H.R. (Calf 1417).	50 mg.	1539	16
H 110. J.B. (a)	50 mg.	1483	40
H 110. J.B. (a)	50 mg.	1491	17
H 110. J.B. (b)	50 mg.	1515	34
H 127. R.R.	50 mg.	1585	48
H 128. D.D.	45 mg.	1567	42
H 128. D.D.	50 mg.	1579	41

H 13. A.D. (Calf 1237): Calf 1327.—Typical of acute infection.

H 49. T.C. (Calf 1263): Calf 1347.—The lungs, spleen, and a thoracic gland are typical of acute infection; the specimens of liver and kidney show less severe disease, but in the latter there is a tubercle containing numerous bacilli.

H 49. T.C. (Original material): Calf 1501.—The tissues are typical of acute infection, but perhaps the number of bacilli in the lungs is slightly below the average.

H 53. D.H. (b): Calves 1507 and 1545.—The lungs of these animals show acute infection with numerous bacilli, but the amount of tissue destruction is below the average. Only slight lesions have been found in the livers and kidneys. The spleens, popliteal glands, and thoracic glands are typical of acute infection.

H 60. W.B. (Bronch. Gl.): Calf 1099.—The lungs, liver, kidneys, and a thoracic gland all show acute disease, typical of that which is produced by highly virulent bacilli.

H 60. W.B. (Bronch. Gl.): Calf 1107.—The lungs show advanced, caseous lesions, containing moderate numbers of bacilli. Fibrin is present. The lesions are obviously progressive; but bacilli are less numerous than is usual in acute cases, a fact which may perhaps be accounted for by the relative smallness of the dose (10 mg.). In the kidneys there are several small lesions which are tending to caseate and contain giant cells; bacilli are scanty. A thoracic gland is almost completely caseous and contains calcareous foci; bacilli are much less numerous than is usual in acute infections.

H 60. W.B. (Bronch. Gl.): Calf 1109.—The lungs are typical of acutely progressive infection; bacilli are numerous and fibrin is present. The liver contains many caseous lesions which are obviously progressive; bacilli are rather scanty. The kidneys contain several tubercles in which bacilli are scanty. A thoracic gland is typical of acute infection and contains numerous bacilli.

H 60. W.B. (Bronch. Gl.): Calf 1425.—The tissues examined (lungs, liver, kidneys, spleen, and a bronchial gland) though showing progressive disease, which is advanced in the lungs, are less severely affected than is usual in infections which terminate fatally within this period. There is a tendency to the formation of fibroblasts at the periphery of the lesions, and bacilli are not very numerous.

H 69. F.K. (Meninges): Calf 1267.—Typical of acute infection.

H 77. R.L.: Calf 1349.—The histological characters of the lesions are typical of acute infection, but bacilli are less numerous than usual.

H 88. E.L.: Calf 1271. — Typical of acute infection.

H 89. W.I. (Lung): Calf 1351. — Typical of acute infection.

H 90. I.P.: Calves 1489 and 1493.—The lungs are crowded with tubercles which are breaking down and contain very numerous bacilli; the lesions are relatively small in Calf 1493. The livers, spleens, and mediastinal glands are also typical of highly acute infection. No bacilli have been found in the kidneys of Calf 1493, but a few are present in Calf 1489.

H 100. R.S. (Calf 1419): Calf 1511.—Typical acute infection. The animal from which the culture used for inoculation was derived experienced a much milder form of disease.

H 100. R.S. (Calf 1419): Calf 1517.—Early miliary tuberculosis. Bacilli less abundant than is usual in acute infections.

H 100. R.S. (Original material): Calf 1523.—Typical of highly acute infection.

H 100. R.S. (*Calf 1409*) *: Calf 1543.*—All the tissues examined are typical of highly acute infection.

H 105. G.S. (*Calf 1449*) *: Calf 1521.*—The lungs are typical of acute infection. The liver is less severely affected; it contains small tubercles in which giant cells are present and bacilli are scanty. The kidneys contain caseous, discrete, but progressive tubercles in which bacilli are numerous. The spleen and an iliac gland show advanced lesions, contain very numerous bacilli, and are typical of acute infection.

H 105. G.S. (*Calf 1405*) *: Calf 1527.*—The lungs are typical of acute infection. Only a few, slight lesions have been found in the liver and none in the specimen of kidney. The spleen and a mediastinal gland are typical of acute infection. A tubercle of progressive type has been found in the right auricle.

H 107. H.H. (*Calf 1453*) *: Calf 1519.*—The lesions are typical of acute infection, being in marked contrast to those of Calf 1453, from which the culture used for inoculation was obtained.

H 108. H.R. (*Calf 1417*) *: Calf 1539.*—The tissues are typical of acute infection, being in marked contrast to those of Calf 1417 from which the culture used for inoculation was obtained.

H 108. H.R. (*Calf 1539*) *: Calf 1509.*—Typical of acute infection.

H 110. J.B.: Calves 1483, 1491, and 1515.—Typical acute infections.

H 127. R.R.: Calf 1585.—Typical of acute infection.

H 128. D.D.: Calves 1567 and 1579.—Typical of acute infection.

(*b*) Strains of Lower Virulence.

Tissues have been examined of the following calves, in which the subcutaneous inoculation of culture or tissue emulsion did not produce fatal or severe disease:—

Virus.	Dose of Bacilli.	Number of Calf Inoculated.	Duration of Experiment in Days.
H 13. A.D. (Calf 301).	100 mg.	1175	81
H 13. A.D. (Calf 301).	50 mg.	1177	110
H 13. A.D. (Calf 301).	100 mg.	1207	92
H 13. A.D. (Calf 1175).	Tissue emulsion.	1237	52
H 49. T.C. (Original material).	50 mg.	1213	80
H 49. T.C. (Calf 1213).	Tissue emulsion.	1263	91
H 53. D.H. (a)	50 mg.	1135	79
H 53. D.H. (a)	50 mg.	1435	94
H 53. D.H. (a)	50 mg.	1477	96
H 53. D.H. (b) (G.P. from original material).	50 mg.	1535	103
H 60. W.B. (Meninges).	50 mg.	989	91
H 60. W.B. (Lung).	50 mg.	1081	90
H 68. R.B.	100 mg.	1161	91
H 68. R.B.	100 mg.	77*	156
H 69. F.K. (Mesenteric Gland).	50 mg.	1249	101
H 72. H.C.	50 mg.	1365	115
H 79. J.N. (Dog 31).	Tissue emulsion.	1243	38
H 85. H.B.	50 mg.	1289	94
H 89. W.I. (Lung).	50 mg.	1275†	105
H 90. I.P. (Retro-peritoneal Gland).	50 mg.	1383	92
H 90. I.P. (Retro-peritoneal Gland).	80 mg.	1471	90
H 92. D.N.	50 mg.	1317	26
H 100. R.S. (G.P. from original material).	100 mg.	1409	119
H 100. R.S. (G.P. from original material).	50 mg.	1419	122
H 105. G.S. (G.P. from original material).	92 mg.	1405	101
H 105. G.S. (G.P. from original material).	48 mg.	1449	130
H 107. H.H. (G.P. from original material	50 mg.	1453	126
H 107. H.H. (G.P. from original material).	80 mg.	1497	104
H 107. H.H. (Calf 1453).	100 mg.	1505	101
H 107. H.H. (Calf 1453).	50 mg.	1513	102
H 107. H.H. (Calf 1497).	50 mg.	1549	97
H 108. H.R. (G.P. from original material).	50 mg.	1417	125
H 108. H.R. (G.P. from original material).	88 mg.	1421	101
H 111. S.E.	50 mg	1429	98
H 111. S.E.	50 mg.	1459	92

* An adult cow. † Above the average age.

H 13. A.D. (*Calf 301*) *: Calf 1175.*—The lung contains discrete, caseous tubercles which are commencing to calcify; they contain few bacilli and are obviously of the chronic type. In the liver there are several suspicious foci which cannot be called definite tubercles; no bacilli have been found. In the kidney there is a small, slightly caseous area in which one bacillus has been found. The spleen contains caseous, circumscribed tubercles; giant cells are also present outside these areas; in some of the lesions there is a moderate number of bacilli. In a popliteal gland several giant cells are present and also small caseous patches without definite boundaries; bacilli are scanty.

H 13. A.D. (*Calf 301*)*: Calf 1177.*—This animal was killed 110 days after inoculation. The lungs contain small, obviously retrogressive, caseo-calcareous lesions which are surrounded by a dense fibrous zone; no bacilli have been found in them. The liver contains a few suspicious foci which are not definite enough to call tubercles; no bacilli have been found. No lesions or bacilli have been found in the specimens of kidney and spleen. A mediastinal gland contains many caseous patches and giant cells; bacilli are scanty.

H 13. A.D. (*Calf 301*) *: Calf 1207.*—The specimen of lung contains several lesions of the chronic, caseous type. In some of these, bacilli are fairly abundant. In the liver there are several suspicious foci which cannot be described as definitely tubercular; no bacilli have been found in them. In the specimen of kidney no lesions or bacilli have been found. The spleen contains small caseous foci in which giant cells are present; bacilli are scanty.

H 13. A.D (*Calf 1175*)*: Calf 1237*—Calf 1237 was inoculated subcutaneously with 20 cc. of an emulsion of the prescapular gland of Calf 1175, and was killed, when very ill, 52 days afterwards. The specimen of lung contains large patches of consolidation which are extensively caseous. The lesions are irregular, ramifying, and apparently progressive, but have a broader cellular periphery than is usual in acute infections; bacilli are scanty. The liver contains many discrete caseous tubercles which have a fairly well defined peripheral zone; bacilli are scanty. Large, extensively caseous tubercles, containing few bacilli, are present in the kidneys. The spleen is very extensively caseous; bacilli are found in moderate numbers in all parts of the specimen. A popliteal gland contains large caseous lesions; bacilli are rather less numerous than in the spleen.

H 49. T.C. (Original material): Calf 1213.—This animal was killed, in fairly good health, 80 days after inoculation. The specimen of lung contains large, irregular patches of consolidation which are partially caseous and contain numerous giant cells; the cellular element is much more abundant than in acute infections, but there is no definite boundary zone at the periphery of the infected areas, and the lesions appear to be slowly progressive; bacilli are scanty. The liver contains many minute, slightly caseous tubercles and also several isolated giant cells; no bacilli have been found. A slightly caseous tubercle, in which no bacilli have been found, is present in the specimen of kidney. In the spleen there are fairly large patches of commencing caseation, containing giant cells; bacilli are scanty. A mediastinal gland contains numerous caseous areas and many giant cells; bacilli are scanty; they sometimes occur within giant cells.

H 49. T.C. (Calf 1213): Calf 1263.—Specimens of lung, liver, kidney, spleen, and a thoracic gland all show caseous tubercles of retrogressive type; no bacilli have been found in them.

H 53. D.H. (a): Calf 1135.—In the specimen of lung there are small lesions of doubtful nature; no tubercle bacilli have been found. No tubercles or bacilli have been found in the specimens of liver or kidneys. In the spleen there is a small, caseous tubercle in which no bacilli have been found. In a bronchial gland there are several small caseous tubercles which are surrounded by fibroblasts and contain a few bacilli.

H 53. D.H. (a): Calves 1435 and 1477.—In the lungs discrete, centrally caseous, and apparently retrogressive lesions are present; bacilli are rare. No lesions or bacilli have been found in the livers, kidneys, and spleens. Caseous lesions of a chronic type, with few bacilli, are present in a suprarenal capsule and a thoracic gland of Calf 1477.

H 53. D.H. (b): Calf 1535.—No lesions or bacilli have been found in the specimens of lungs, liver, kidneys, and spleen. In a bronchial gland there are several tubercles of chronic type in which bacilli are scanty. In one portion of the udder (right fore) there are several small tubercles of chronic type. No bacilli have been found in them.

H 60. W.B. (Meninges): Calf 989.—The lungs contain lesions of a chronic type. Bacilli are fairly numerous in some of the caseous patches.

H 60. W.B. (Lung): Calf 1081.—Lesions of a chronic type are present in the lungs and contain giant cells; bacilli are rare; the lesions are larger than those usually produced by slightly virulent viruses after this method of inoculation. In the liver there are some small suspicious foci in which no bacilli have been found. In the kidneys there is some interstitial change which is probably not tubercular; no bacilli have been found. In a thoracic gland no lesions or bacilli have been found.

H 68. R.B.: Calf 1161.—Small, obviously retrogressive tubercles are present in the lungs; no bacilli have been found in them. Neither bacilli nor definite tubercles have been found in the specimens of liver, kidneys, and a bronchial gland.

H 68. R.B.: Cow 77.—Neither tubercles nor tubercle bacilli have been found in the following specimens:—The four quarters of the udder (2 different pieces of each), the lungs, liver, kidneys, spleen, and suprarenal capsule (one specimen of each).

H 69. F.K. (Mesenteric Gland): Calf 1249.—The lungs contain large caseous patches which have a well defined boundary zone, contain numerous giant cells, and in general appearance are chronic rather than acute in type; bacilli are scanty. The lesions in the liver, and also in a lumbar gland, are of similar character. In caseous patches present in the kidney no bacilli have been found.

H 72. H.C.: Calf 1365.—In the lungs no definite lesions or bacilli have been found. In the liver there are a few suspicious foci containing giant cells, but no bacilli have been found in them. No lesions or bacilli have been found in the kidneys or spleen. In an iliac gland there are a few tubercles of chronic type in which bacilli are rare.

H 79. J.N.: Calf 1243.—Calf 1243 was inoculated subcutaneously with an emulsion of the spleen of Dog 31 and was killed 38 days afterwards. The lungs show thickening of the alveolar walls and many small consolidated patches; no tubercle bacilli have been found in these areas and they do not possess the structure of definite tubercles, though it is possible that some of them may have been caused by tubercle bacilli which have disappeared. In the liver there are several small, suspicious foci, in which no tubercle bacilli have been found No lesions or bacilli have been found in the kidneys. In the spleen there are no definite lesions, but one tubercle bacillus has been found. A mesenteric gland contains several giant cells; no bacilli have been found. A mediastinal gland contains a small caseous patch in which tubercle bacilli are scanty. In a popliteal and a prepectoral gland no lesions or bacilli have been found. The prescapular gland on the side of inoculation is extensively caseous; the bacilli are long and beaded and not very numerous. In the heart muscle there is a suspicious focus of cellular infiltration, in which no bacilli have been found. No lesions or bacilli have been found in a popliteal and a prepectoral gland.

H 85. H.B.: Calf 1289.—No lesions or bacilli have been found. The spleen and a mediastinal gland contain large caseo-calcareous tubercles in which no bacilli have been found.

H 89. W.I. (Lung): Calf 1275.—In contrast to Calf 1351 Calf 1275, though inoculated with 50 mg. of the same virus, developed relatively slight disease and was killed 105 days after inoculation. It must be observed, however, that Calf 1275 was 18 weeks old when inoculated, whereas Calf 1351 was only six weeks old. The specimen of lung shows numerous lesions of the chronic type; they are over-run with small lymphocytes and contain giant cells; leucocytes are scanty, and bacilli are rare. The liver shows chronic lesions which contain giant cells and are undergoing fibroid change both at their margins and in the interior; no bacilli have been found. Small, chronic lesions, in which no bacilli have been found, occur in the kidneys. A thoracic gland contains chronic lesions in which bacilli are scanty.

H 90. I.P.: Calf 1383.—The lungs, liver, and spleen show retrogressive, caseo-calcareous tubercles in which no bacilli have been found. In a mediastinal gland there are caseous tubercles of a chronic type in which bacilli are very rare.

H 90. I.P.: Calf 1471.—In the specimens of lungs, liver, kidneys, and spleen no lesions or bacilli have been found. In a bronchial gland there are caseous tubercles in which bacilli are rare.

H 92. D.N.: Calf 1317.—This animal died from intercurrent disease, 26 days after inoculation. The lungs contain minute lesions which are retrogressive in character but contain a few bacilli. The liver contains no definite tubercles, and no tubercle bacilli have been found in it. The specimen of kidney shows interstitial nephritis but contains neither tubercles nor tubercle bacilli. Two giant cells, but no tubercle bacilli, have been found in the spleen. Collections of blue bacilli occur in several of the specimens, including a lumbar gland.

H 100. R.S. (Original material): Calf 1409.—In the lungs there are moderately large areas showing slight caseation. The lesions are quiescent in character, though perhaps not definitely retrogressive, and contain only a few bacilli. In the liver there are a few suspicious foci but no definite tubercles and no bacilli. No lesions or bacilli have been found in the kidneys or spleen. In a bronchial gland there are chronic, caseous tubercles in which bacilli are scanty.

H 100. R.S. (Original material): Calf 1419.—In the lungs, liver, spleen, and a bronchial gland, there are large, discrete tubercles which are chronic or retrogressive in character and contain few bacilli. No lesions or bacilli have been found in the kidneys or spleen.

H 105. G.S. (Original material): Calf 1405.—In the lungs there are extensive, ramifying lesions which have undergone fibro-calcareous change; no bacilli have been found. In the liver there are many small, retrogressive tubercles filled with lymphocytes and leucocytes and containing giant cells; no bacilli have been found. Similar lesions are present in the kidneys. The spleen contains large calcareous lesions of a chronic type; bacilli are very scanty. In a mediastinal gland there are extensive fibro-calcareous tubercles resembling those found in the lungs. A haemolymph gland shows calcareous tubercles in which no bacilli have been found.

H 105. G.S. (Original material): Calf 1449.—The lungs contain a few small, retrogressive tubercles in which no bacilli have been found. No lesions or bacilli have been found in the specimens of liver, kidneys, and spleen. A popliteal gland contains a large caseo-calcareous tubercle in which bacilli are rare.

H 107. H.H. (Original material): Calf 1453.—In the lungs there is a large retrogressive tubercle showing marked fibroid change; no bacilli have been found in it. No lesions or bacilli have been found in the specimens of liver, kidneys, spleen, and a thoracic gland.

H 107. H.H. (Original material): Calf 1497.—No lesions or bacilli have been found in the specimens of lungs, liver, kidneys, and spleen. In a mediastinal gland there are caseous tubercles of a chronic type in which bacilli are scanty. In the muscle of the right auricle there is a small tubercle which is infiltrated with lymphocytes and is evidently retrogressive, though a few bacilli have been found in it.

H 107. H.H.: Calves 1505, 1513, and 1549.—No lesions or bacilli have been found in the specimens of Calf 1505. In Calf 1513 suspicious foci, with no bacilli, have been found in the lungs; neither lesions nor bacilli have been found in the liver, kidney, and spleen; chronic lesions, with scanty bacilli have been found in a mediastinal gland and in the right auricle. In Calf 1549 chronic lesions, containing few bacilli, have been found in the lungs, liver, right auricle, udder, and a mediastinal gland.

H 108. H.R. (Original material): Calves 1417 and 1421.—In specimens of lungs, liver, kidneys, and spleen of the two animals neither lesions nor bacilli have been found. A popliteal gland of the former and a bronchial gland of the latter show chronic tubercles in which bacilli are rare.

H 111. S.E.: Calves 1429 and 1459.—In the former animal the lungs show a few minute foci filled up with lymphocytes, and there are some suspicious foci in the spleen; no bacilli have been found. The latter animal shows acute nephritis, not associated with tubercle bacilli. In the lungs there are a few tubercle bacilli but no definite lesions.

General Results.

The histological details of these experiments upon calves correspond with those already recorded in my previous Report on the lesions produced in calves inoculated subcutaneously with large doses for the purpose of emphasising differences in bacterial virulence. The inoculations followed by rapidly fatal effects have caused lesions which, with a few slight exceptions, are typical of this acute type of tuberculosis. They include three calves inoculated with the sputum viruses H 127. R.R. and H 128. D.D.

The inoculations with less virulent material have, as rule, produced lesions relatively few in number, small in size, and chronic or retrogressive in character. But in some cases more severe disease has been produced. Calf 1237 (H 13. A.D.), for example, shows extensive disease of the lungs and the general condition of this and other organs indicates a degree of infection intermediate between the acute and the chronic. Calf 1213 (H 49. T.C.) also exhibits lesions of an intermediate type, the disease being perhaps not quite so severe as in Calf 1237.

Special attention may be called to the irregularity of the results obtained with certain viruses.

H. 49. T.C. Two calves (1213 and 1501) were inoculated each with 50 mg. of culture (from original material); in the latter the disease was definitely acute; in the former it was less acute and may be described as being of intermediate severity. A tissue emulsion from Calf 1213 produced only a mild type disease in Calf 1263; but 50 mg. of culture from Calf 1263 produced acute disease in Calf 1347. The dose of bacilli contained in a tissue emulsion is much less than in 50 mg. of culture, and it is known that tissue emulsions containing highly virulent bacilli do not necessarily produce severe disease in calves; there is therefore nothing particularly unusual about the mildness of the infection in Calf 1263. Omitting this case, the amount of discrepancy in the other results is only slight, and may be summed up by saying that the virulence of H. 49. T.C. is high, but less uniformly high than that of a typical bovine virus.

H 53. D.H. Material from the patient yielding this virus was obtained on two separate occasions. The first material (*a*) yielded bacilli possessing relatively low virulence for the bovine (Calves 1135, 1435, and 1477). The second material (b), obtained 3 years and 8 months later, proved to be of high bovine virulence in two instances (Calves 1507 and 1545), and of low bovine virulence in another (Calf 1535).

H 60. W.B.—The bacilli from the bronchial gland of the human subject yielding this virus were of high bovine virulence (Calves 1099, 1107, and 1109); but bacilli from other tissues of the same case (meninges and lungs) were of low virulence for the bovine.

H 69. F.K.—Bacilli derived from the human meninges of this case produced acute disease (Calf 1267); but the bacilli from the mesenteric gland produced only mild infection (Calf 1249).

H 89. W.I.—This virus produced severe disease in one animal (Calf 1351) and mild disease in another (Calf 1275), which was, however, 12 weeks older.

H 90. I.P.—Two calves (1383 and 1471) inoculated with bacilli obtained from a retro-peritoneal gland of the human subject showed only slight disease, but a culture from the former calf produced severe disease in Calf 1489, and a culture from Calf 1471 produced severe disease in Calf 1493.

H. 100. R.S.—In Calves 1409 and 1419 lesions of a chronic type were produced by cultures from the original material; but in Calf 1523 culture from the same source produced acute infection. A culture from Calf 1409 produced acute infection in Calf 1543; and a culture from Calf 1419 produced the same result in Calf 1517.

H 105. G.S.—Cultures from original material produced mild infection in Calves 1453 and 1497. A culture from Calf 1497 produced disseminated lesions of chronic type in Calf 1549. A culture from Calf 1453 produced only slight infection in Calves 1505 and 1513 but typically acute disease in Calf 1519.

H 108. H.R.—Cultures from original material produced only slight infection in Calves 1417 and 1421. But a culture from Calf 1417 produced typically acute infection in Calf 1539; and a culture from Calf 1539 produced a similar result in Calf 1509.

(c) Additional Intravenous Inoculations.

(1.)

VIRUS—H 53. D.H.

Animal Inoculated.—Calf 1391.

Calf 1391 was inoculated intravenously with 1 mg. of culture of H 53. D.H. (*a*), and was killed, when moribund, 40 days afterwards.

Lungs.

The specimen of lung is almost completely solidified. There are several small patches of commencing caseation, but the amount of tissue destruction is less than after typical infection with highly virulent bacilli. Bacilli are very abundant.

Liver.

No tubercles or bacilli have been found.

Kidneys.

There is one small focus in which a few bacilli are deposited.

Spleen.

One minute lesion, containing one bacillus, has been found.

Mediastinal Gland.

There are many caseous areas in which bacilli are very plentiful.

SUMMARY.

The severe infection of the lungs is sufficient to account for death ; most of the bacilli seem to have been limited to the thorax.

(2.)

VIRUSES—H 81. P.W. and H 83. G.C.

Animal Inoculated.—Cow 61.

Cow 61 was inoculated (1) subcutaneously with 100 mg. of culture of H 81. P.W., and (2) 90 days later, intravenously with 150 mg. of culture of H 83. G.C. The animal was killed, in good health, 529 days after the second inoculation.

Lungs.

The specimen contains many caseous tubercles which are of various sizes and are collected in groups. The periphery of the tubercles is vascular, shows a broad zone of lymphocytes, and contains relatively little fibrous tissue. Bacilli occur in most of the lesions in moderate numbers, and are not confined to the central areas of caseation.

Liver.

No tubercles have been found, but there is a small group of bacilli within one of Glisson's capsules.

Spleen.

There are no lesions in the specimen examined, but two bacilli have been found.

Mediastinal Gland.

No tubercles have been found, but there is one fairly large group of bacilli.

Supramammary Gland.

The gland is very extensively caseous. Bacilli are rather scanty.

Udder.

Specimens from each of the four quarters have been examined and have all been found to contain tubercles. The tubercles vary in size from small lesions at the commencement of caseation to large and almost completely caseous lesions. Some of the tubercles are centrally calcareous. Fibrous elements and plasma cells are abundant at the periphery of the lesions. In most of the lesions tubercle bacilli are very numerous. In addition to abundant groups of bacilli in the caseous areas there are, in many situations, large numbers of intracellular bacilli in the peripheral zones.

SUMMARY.

The udder contains very large numbers of bacilli which have produced caseous tubercles of a chronic type. The lungs contain many caseous tubercles. Bacilli, but no tubercles, have been found in the specimens of liver, spleen, and a mediastinal gland.

(3.)

VIRUS—H 85. H.B.

Animal Inoculated.—Calf 1373.

Calf 1373 was inoculated intravenously with 10 mg. of culture of H 85. H.B. (from original material), and was killed, when moribund, 28 days afterwards.

Lungs

There are large patches of consolidation which are crowded with leucocytes; they are not caseous and not typical of tubercular pneumonia. Tubercle bacilli are not uniformly distributed but are numerous in many places and are evidently multiplying.

Liver.

The liver contains numerous progressive, necrotic areas in which bacilli are rather scanty.

Kidneys.

There are many small patches showing an interstitial infiltration of lymphocytes, leucocytes, and plasma cells. In these areas bacilli are fairly plentiful.

Spleen.

Bacilli are distributed about the tissue in rather scanty numbers; there are a few very minute caseous patches, but in most places the bacilli have produced no tissue change.

Mediastinal Gland.

Bacilli are very plentiful. There are patches of slight caseation, but bacilli often occur in large numbers, both in the cortical and medullary areas, in situations where there is very little tissue change.

SUMMARY.

The lungs are in a condition of pneumonia and contain tubercle bacilli in large numbers. In other organs bacilli are present but relatively scanty.

II.—LESIONS IN RABBITS.

(*a*) Lesions Produced by Various Viruses.

Rabbits Inoculated with Human Viruses.

Virus Inoculated.	Dose.	Mode of Inoculation.	No. of Rabbit.	Duration of Experiment in Days.*
H 49. T.C. (Original material).	·1 mg.	Intravenous.	1181	D 31
H 75. A.H.	1 mg.	Intravenous.	1205	K 101
H. 77. R.L.	·1 mg.	Intravenous.	1246	D 23
H 78. O.D.	1 mg.	Intravenous.	1212	D 28
H. 78. O.D.	1 mg.	Intravenous.	1215	D 18
H 79. J.N.	Tissue Emulsion.	Intraperitoneal.	1175	K 93
H 79. J.N.	1 mg.	Intravenous.	1748	D 27
H 85. H.B.	·1 mg.	Intravenous.	1496	D 48
H 87. W.D.	10 mg.	Intravenous.	1342	D 26

* K = Killed. D = Found dead.

H 49. T.C. (Original material): Rabbit 1181.—The specimen of lung is almost completely solidified and exhibits irregular patches of caseation. In some of these there are large masses of bacilli but in the majority bacilli are not very numerous. The specimen is typical of advanced tubercular pneumonia and is acute in type; perhaps there is rather less necrosis than would be met with in very acute infection. In the liver, tubercular foci are distributed throughout the tissue in large numbers. They have no limiting zone of cells, but contain numerous giant cells and are less necrotic than is usual in highly acute infection. Bacilli are scanty. Numerous tubercles are present in the kidneys, and some of them are commencing to caseate. Except in the caseous foci, bacilli are not numerous. A bronchial gland is extensively caseous and contains large numbers of bacilli.

H 75. A.H.: Rabbit 1205.—The specimen of lung is nearly completely solidified and contains extensive patches of caseation; within the caseous areas bacilli are found in fairly large numbers, but are scattered about singly and not growing into groups; amongst the cellular periphery of the lesions bacilli are found but are less numerous; the general characters of the specimen indicate infection of a slowly progressive type. The liver contains a few suspicious foci but no definite tubercles; no bacilli have been found. In the specimen of kidney there is a large caseous lesion, in the centre of which bacilli have multiplied very abundantly. No lesions or bacilli have been found in the spleen.

H 77. R.L.: Rabbit 1246.—The lungs are packed with miliary tubercles which are centrally caseous, are becoming confluent, and contain large numbers of bacilli. A few bacilli have been found within blood-vessels. The liver is riddled with caseating miliary tubercles containing numerous bacilli. In the kidneys there are numerous tubercles, which are less advanced than those in the lungs and liver and contain fewer bacilli. They are invaded with lymphocytes and leucocytes and are only slightly caseous. They appear to have originated in the interstitial tissue between the tubules. Bacilli are distributed throughout the spleen in large numbers and have produced areas of early caseation. The heart muscle (left ventricle) and a thigh muscle show small deposits of bacilli between the muscle fibres. The bacilli have led to partial disappearance of the muscle substance, the formation of fibroblasts, and an infiltration of lymphocytes. In the marrow of a thigh bone bacilli are abundant and have produced early caseation.

H 78. O.D.: Rabbit 1212.—The lungs are crowded with semi-confluent, miliary, caseous tubercles, in which bacilli are fairly numerous, but much less numerous than after intravenous inoculation with highly virulent bacilli; bacilli are also present in all parts of the tissue apart from the tubercles. In the liver there are several small foci which are not definite enough to be called tubercles but consist of small collections of lymphocytes and leucocytes amongst slightly disintegrated liver cells; bacilli are found in some of these situations but are rare. In the kidney there are small, slightly caseous tubercles containing few bacilli. A bronchial gland contains some small groups of bacilli and shows early caseation.

H 78. O.D.: Rabbit 1215.—The tissue of the lungs is almost completely solidified. Caseation is commencing in all parts of the section but has not progressed very far, and the total amount of tissue destruction is not great. Bacilli are present in all parts of the tissue but generally occur singly. They have evidently not been able to multiply freely. Bacilli are present in small numbers in all parts of the liver. They have caused partial necrosis of the parenchymatous cells, a swelling and increase of the endothelial elements, and an infiltration of leucocytes. Giant cells are very numerous. Many of them appear to have been formed in the substance of the partially disintegrated parenchymatous cells. In the kidneys there are numerous small patches of interstitial infiltration in which tubercle bacilli are present in scanty numbers. A bronchial gland contains many caseous areas in which bacilli are fairly numerous.

H 79. J.N.: Rabbit 1175.—This animal was killed 93 days after intraperitoneal inoculation with a tissue emulsion from a guinea-pig inoculated with the human lung. The lungs show partial consolidation, irregular in distribution and containing caseous patches; in a few places, in the older parts of the lesions, the bacilli have grown into small clumps, but elsewhere bacilli are scanty. In the liver there are many minute, slightly caseous tubercles containing giant cells; bacilli are rare. In the medullary part of the kidney there is a patch of non-caseous cellular infiltration containing a few bacilli. The spleen contains a few patches of very slight caseation; in some of these one or two bacilli are found.

H 79. J.N.: Rabbit 1748.—The lungs are congested and contain many small, non-caseous patches of consolidation; in these areas bacilli are very abundant and have sometimes assumed a rosette formation. The liver is swarming with bacilli, which have produced numerous rosette formations strongly suggestive of the avian bacillus. In the kidneys there are a few small clusters of bacilli, which again have a tendency to assume the rosette form. The spleen is very densely crowded with bacilli, forming abundant colonies and rosettes. The resemblance to the avian type of infection in the tissues of this animal is very strange.*

H 85. H.B.: Rabbit 1496.—The lung is almost completely solidified and contains some caseous patches. Bacilli are very abundant in these caseous areas, but in the rest of the tissue, though fairly numerous, they are much less abundant than in typical highly acute infection; some bacilli are present within blood-vessels; leucocytes are very abundant and are collected round the groups of bacilli. The liver contains many caseous patches, obviously acute in type, and tending to become confluent; they contain numerous bacilli; though the infection is undoubtedly acute, the liver does not show very advanced necrotic changes. The kidneys contain many caseating lesions; they have an abundant cellular periphery but contain numerous bacilli and are evidently progressive. The spleen contains numerous semi-confluent caseous areas in which bacilli are plentiful. A bronchial gland is very extensively caseous and contains enormous numbers of bacilli.

H 87. W.D.: Rabbit 1342.—The lungs show great thickening of the alveolar walls and contain many patches of incomplete consolidation, formed by the partial amalgamation of circular, slightly caseous tubercles; within the tubercles bacilli occur in small numbers, but where the thickened alveolar walls do not show definite tubercle formation, bacilli are only found occasionally. The liver shows small caseous tubercles and minute foci of cellular infiltration which have not become caseous; bacilli are present in the lesions in small numbers. The kidneys contain many small lesions which are not caseous but contain numerous wandering cells, the lymphocytes being in excess of the leucocytes; in these lesions bacilli are numerous; it is unusual to find bacilli so plentiful in lesions of this type which do not show caseous degeneration.

General Results.

The above histological details illustrate different degrees of severity of the infective process in rabbits, particularly after intravenous inoculation.

Thus, H. 77. R.L., which is equal in virulence to a bovine virus, in an intravenous dose of 0·1 mg. of culture produced death in 23 days with typically acute disease and abundant multiplication of bacilli. With the same intravenous dose H 49. T.C. and H. 85. H.B. produced death in 31 and 48 days, respectively; the infections may be described as acute, but there is rather less tissue destruction and rather less abundant multiplication of bacilli than in very acute cases. With the larger intravenous dose of 1 mg. H 78. O.D. produced death in 18 days in one rabbit and death in 28 days in another; there is extensive disease, particularly in the lungs of the two animals, but the multiplication of bacilli is distinctly less than with more virulent viruses. In Rabbit 1205, after the same intravenous dose of H. 75. A.H., the duration of life was much longer; the animal was killed 101 days after inoculation and then showed extensive disease of the lungs of a slowly progressive type.

(b) Early Stages of the Infective Process after Intravenous Inoculation.

Dr. A. S. GRIFFITH inoculated a series of rabbits (Rabbits 1567–71) with a culture of human bacilli (H 90. I.P.—*Strain*:—G.P. from Retro-peritoneal Gland.) The inoculations were made intravenously with a dose of 1 mg. One animal was killed at each of the following periods after inoculation:—24 hours, 3 days, 5 days, 8 days, and 14 days.

Lungs.

24 hours.—The lung capillaries are slightly swollen and a few small clumps of bacilli are found entangled in them. Isolated bacilli are also occasionally found in the alveolar walls. Small lymphocytes are slightly in excess of the normal. Leucocytes are more definitely in excess and often occur in small groups. Though some of the deposits of bacilli are not associated with leucocytes, these cells are often found collected in the neighbourhood of bacilli and sometimes contain bacilli in their interior.

3 days.—There is increased congestion of the capillaries. Deposits of bacilli occur in twos and threes, and, though not abundant, are rather less scanty than in the former specimen. Where the bacilli occur, the endothelial and epithelial cells are often swollen and stain less sharply than normal, an appearance which can be detected with a low power. Lymphocytes are in excess of the normal, but leucocytes are not numerous, being distinctly fewer than in the former specimen. A few bacilli have been found within leucocytes.

5 days.—The general thickening of the alveolar walls is more pronounced, and there is a catarrhal exudate in many of the alveoli. There is also a general increase in the number of bacilli. Leucocytes are more numerous than in the last specimen and are generally distributed, both in proximity to the bacilli and elsewhere; a few bacilli have been found actually within leucocytes. The swelling and partial disintegration of the endothelial and epithelial cells is more marked than in the last specimen. There is no increase in the number of lymphocytes. Many of the bacilli have increased in length and in several places there is evidence of multiplication.

8 days.—The lesions have expanded and are more numerous. Their centre consists of swollen and partially disintegrated epithelial and endothelial cells with, sometimes, the addition of a few leucocytes. At the periphery there is a zone of leucocytes and lymphocytes, the latter cells being more numerous. The bacilli have increased in number; a small proportion of them have been found within leucocytes.

14 days.—There is no increase in the lesions as compared with the last specimen, and the number of bacilli which they contain shows a diminution. Small lymphocytes have increased in number and are present in the interior as well as at the periphery of the lesions. Leucocytes are scattered about the lesions in small numbers; a few have been found containing bacilli.

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* I have called Dr. A. S. Griffith's attention to this result. It appears that in a calf inoculated with H 79. J.N. a suspicious focus was found in a mesenteric gland. From this focus an avian bacillus was isolated and was inoculated into Rabbit 1748.

Livers.

24 hours.—About half a dozen deposits of one or two bacilli have been found. Most of these are contained within, or closely applied to, liver cells, the nuclei of which are distorted and stain more deeply and diffusely than usual. In a few of the cases, the adjacent endothelial cells show similar changes of their nuclei. Two bacilli have been found within leucocytes.

3 days.—There is no increase in the number of bacilli, which are rare and difficult to find. In one place two bacilli have been found in a small area consisting of the fusion of a few endothelial cells and liver cells ; the nuclei of these cells are distorted and stain diffusely. In another place there is a similar but rather larger area containing a few bacilli ; into this area several small lymphocytes have penetrated but no leucocytes. Very few leucocytes have been found attacking bacilli.

5 days.—Bacilli are very scanty ; a few have been found within endothelial cells and within liver cells. A few of the endothelial cells containing bacilli show multiplication of their nuclei by direct division. Leucocytes are not numerous and show no relationship to the bacilli.

8 days.—Bacilli are distributed about the tissue in greater numbers than in former specimens, and have produced minute lesions which are recognisable under a low power. In most of these lesions bacilli are scanty (three or four in each), but in a few they are more numerous and show signs of having multiplied ; as many as 60 bacilli have been counted in one lesion the size of the microscopic field (oil-immersion lens). The lesions are formed by a fusion of a few contiguous liver cells and endothelial cells and a multiplication, by direct division, of the nuclei of both these types of cells but mainly of the endothelial cells. In the smaller lesions, containing very few bacilli, the increase of nuclei is only slight, but the largest lesions are filled with nuclei derived from the sources mentioned. A few small lymphocytes are present in some of the lesions. Leucocytes are nearly always present, but in variable numbers, not exceeding about a dozen in the largest lesions. In the lesion containing about 60 bacilli some of the leucocytes contain bacilli and some broken down leucocytes are also present amongst the bacilli. There are a few cells which give a slight reaction to Pappenheim's stain, but no unmistakable plasma cells have been found.

14 days.—There are numerous lesions which are larger than those in the last specimen and are characterised by a slight disintegration of the epithelial and endothelial cells and a tendency to the multiplication of their nuclei by direct division. These cells also show, occasionally, a mitotic figure. Bacilli are scattered about the lesions in small numbers. Groups, indicating recent division, are rarely found. Small lymphocytes have invaded the lesions in larger numbers than in the last specimen, whereas leucocytes are rarer and none have been found containing bacilli.

Kidneys.

A few bacilli have been found in each of the specimens (about half a dozen in each section). They occur in the interstitial tissue, with the exception of one found in a blood-vessel, and are not associated with any definite lesions.

Spleens.

24 hours.—Bacilli are scattered about the tissue in small numbers and generally occur singly. Leucocytes are very abundant, but very few have been found which contain bacilli.

3 days.—Bacilli are present in scanty numbers. There is no increase as compared with the last specimen, but a few of them have become longer. Leucocytes are very numerous, though not quite so abundant as in the last specimen ; they are more frequently found in close proximity to bacilli, and a few have been found with bacilli in their interior.

5 days.—There is no appreciable increase in the number of bacilli, and no more have been found within leucocytes ; these cells are not quite so numerous as in the last specimen.

8 days.—Bacilli are scattered about singly and show no appreciable increase in number. They are often associated with a swelling up of the nuclei of endothelial cells. There are many large groups of leucocytes, but no bacilli have been found contained within these cells.

14 days.—The bacilli show only slight increase in numbers, but in many of the places where they occur the nuclei of endothelial cells are swollen and have multiplied by direct division ; these areas also show a slight tendency to caseation. Within these lesions leucocytes are almost entirely absent, but in the surrounding tissue they are numerous.

SUMMARY.

This series of cases illustrates a much milder type of infection than has been found in two corresponding series of rabbits inoculated with highly virulent bacilli.

(*c*) Early Stages of the Infective Process after Subcutaneous Inoculation.

Dr. A. S. GRIFFITH inoculated a series of rabbits subcutaneously, over the scapula, each with a dose of 50 mg. of the culture used in the preceding series. One animal was killed at each of the following intervals after inoculation :—24 hours, 3 days, 6 days, 8 days, and 14 days.

Twenty-four Hours.

In the local lesion (Rabbit 1572) there is a copious invasion of leucocytes, which have ingested the bacilli in large numbers. Many of the leucocytes in the centre of the mass of culture have lost all their granules and their nuclei stain faintly, but at the periphery of the lesion the supply of fresh leucocytes is very abundant. A few bacilli have made their way between the muscle fibres. No bacilli have been found within blood-capillaries.

No bacilli have been found in the specimens of lung, liver, kidney, and spleen.

Three Days.

In the centre of the local lesion (Rabbit 1573) some of the bacilli are broken up, a few have lost their acid-fast properties, and there is a great increase in the number of the disintegrated leucocytes. At the periphery, both beneath the skin and where the lesion borders on the muscle, the blood-capillaries are numerous and dilated ; their endothelial cells are swollen, and many leucocytes are seen passing out through the endothelial wall. No bacilli have been found within blood-vessels.

No bacilli have been found in the specimens of lung, liver, kidney, and spleen.

Six Days.

In the local lesion (Rabbit 1574), which is much larger than in the 3 days' specimen, the bacilli show no diminution in numbers, and the central parts of the lesion are more necrotic. Leucocytes are still pouring into the lesion, and at the margins there are large numbers of these cells which retain their granular stain. There is also a sprinkling of lymphocytes. At the periphery, both in the loose connective tissue and where the lesion is invading the muscle, there is a large increase in the fixed tissue cells, and many of these cells contain bacilli.

In these outlying regions the proportion of leucocytes which contain bacilli is relatively small. No bacilli have been found within blood-vessels.

No bacilli have been found in the specimens of lung, liver, kidney, and spleen.

Eight Days.

In the necrotic portions of the local lesion (Rabbit 1575) there appears to be some falling off in the numbers of bacilli. At the margins there is some increase in the number of lymphocytes. Many of the fixed cells contain bacilli, but the bacilli do not seem to have multiplied in these situations. No bacilli have been found within blood-vessels.

No bacilli have been found in the specimens of lung, liver, kidney, and spleen.

Fourteen Days.

In the local lesion (Rabbit 1576) there is a further diminution in the number of bacilli. Except in the areas of advanced caseation, there is a dense infiltration of small lymphocytes, together with a few plasma cells. This increase in the invasion of lymphocytes is the most notable change as compared with the preceding specimen. The leucocytes are still numerous and often occur in groups, but their total number is perhaps rather less than in the previous specimen. They are scattered all over the specimen, and some leucocytes, showing a well marked granulation, have made their way into the older necrotic foci. No bacilli have been found within blood-vessels.

No bacilli have been found in the specimens of lung, liver, kidney, and spleen.

SUMMARY.

No disseminated lesions have been found.

(*d*) Lesions Produced by Dead Bacilli.

VIRUS—H 74. D.S.

Animal Inoculated.—Rabbit 1195.

Rabbit 1195 was inoculated intravenously with 50 mg. of dead culture of H 74. D.S., and was killed 21 days afterwards.

Lungs.

There is a slight generalised thickening of the alveolar walls but there are no definite tubercles. In several places a few bacilli are present. They are generally within epithelial or endothelial cells, but the only tissue changes they have caused are a slight swelling up and partial disintegration of these cells and the attraction of a few lymphocytes and leucocytes. In one situation some bacilli have been found in the endothelial cells of a small artery.

Liver.

Bacilli are numerous and are generally within the walls of, or immediately external to, small veins and capillaries. There are many giant cells in the centres of which bacilli are numerous. There is one caseous tubercle with well defined fibrous walls; in this no tubercle bacilli have been found.

Kidneys.

There are no lesions and only two bacilli have been found. They are within a glomerulus.

Spleen.

Bacilli are numerous in all parts of the tissue and often occur in small clumps. They have caused very little alteration in the tissue but are often surrounded by groups of oxyphil leucocytes.

Bronchial Gland.

A few bacilli are present, but have caused no appreciable tissue change.

SUMMARY.

Bacilli are generally distributed, but have caused only slight tissue reaction. Compared with Rabbit 1283, inoculated intravenously with 50 mg. of dead bovine bacilli, the lungs of Rabbit 1195 show less indication of tubercle formation; the livers of the two animals resemble each other; the spleen of Rabbit 1195 contains more bacilli than the corresponding organ of Rabbit 1283.

III.—LESIONS IN MONKEYS AND CHIMPANZEES.

VIRUS—H 8. S.C.

(1.)

Animal Inoculated.—Monkey 67.

Monkey 67 was inoculated subcutaneously with ·01 mg. of culture of H 8. S.C., and was killed 199 days afterwards.

Lungs.

Adjacent to normal lung tissue there is a large consolidated patch which is partially caseous, contains numerous giant cells, and is not separated by any fibrous zone from the surrounding tissue. It contains a few tubercle bacilli.

Liver.

There are a few tubercular foci which are slightly caseous and are surrounded by lymphocytes and fibroblasts. Tubercle bacilli are present but rare.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Large caseous lesions are present. Though chronic in type and containing giant cells, they are not definitely retrogressive.

SUMMARY.

Disseminated tuberculosis of a mild type. Apparently the lesions in the lungs and spleen, though chronic in character, are slowly progressive.

(2.)

Animal Inoculated.—Monkey 69.

Monkey 69 was inoculated subcutaneously with ·1 mg. of culture of H 8. S.C., and was killed 126 days afterwards.

Lungs.

The specimen examined is completely solid and to a large extent caseous. Bacilli are scanty.

Liver.

Discrete tubercles, both large and small, are present. They are of the chronic type and contain very few bacilli. The larger are extensively caseous.

Kidneys.

The specimen contains a circumscribed, caseous tubercle, in which only one tubercle bacillus has been found.

Axillary Gland.

The gland is almost entirely caseous. Bacilli, though not abundant and not occurring in groups, are more numerous than in the other organs.

SUMMARY.

Progressive tuberculosis, of a chronic type.

(3.)

Animal Inoculated.—Monkey 71.

Monkey 71 was inoculated subcutaneously with 1 mg. of culture of H 8. S.C., and was killed 190 days afterwards.

Lungs.

There is a considerable thickening of the alveolar walls which is not suggestive of tuberculosis. Small areas of consolidation also occur which are slightly caseous and are surrounded with lymphocytes and leucocytes. Bacilli are very scanty.

Liver.

The liver contains tubercles of a chronic type, surrounded by lymphocytes and fibroblasts. In one of these tubercles bacilli are fairly numerous, but in many of the lesions very few bacilli are to be found.

Kidneys.

There are a few discrete tubercles which are slightly caseous and are surrounded by a broad zone of lymphocytes and plasma cells. No bacilli have been found.

Spleen.

The spleen is extensively caseous. Bacilli are scanty.

SUMMARY.

Disseminated lesions of a chronic type.

VIRUS—H 53. D.H.

(1.)

Animals Inoculated.—Monkeys 93, 101, and 103.

These three animals were inoculated subcutaneously with culture of H. 53. D.H. (*a*). Monkeys 93 and 101 each received 1 mg.; Monkey 103 received 10 mg. Monkey 93 died 105 days afterwards; Monkey 101 died 42 days after inoculation; and Monkey 103 died 23 days afterwards.

Lungs.

In the lungs of Monkey 93 there is some thickening of the alveolar walls, but no tubercles or bacilli have been found. In the lungs of Monkey 101 the greater part of the tissue is in the same condition, but one slightly caseous tubercle has been found, containing several bacilli. The specimen of the lungs of Monkey 103 is almost normal; it contains neither tubercles nor bacilli.

Livers.

In the livers of all three animals there are some small suspicious foci, but neither definite tubercles nor bacilli have been found.

Kidneys.

In the kidneys of all three animals neither tubercles nor bacilli have been found.

Spleens.

In the spleen of Monkey 93 no lesions or bacilli have been found. The spleen of Monkey 101 contains several slightly caseous foci; in most of them bacilli are either scanty or absent; a larger lesion is also present which is not caseous but contains large numbers of leucocytes and also cells resembling fibroblasts; in this lesion bacilli are very abundant; a lesion rather smaller than the last mentioned, but also showing a tendency to fibroid change, contains moderate numbers of bacilli. The spleen of Monkey 103 contains several small, slightly caseous patches in which bacilli are fairly numerous; bacilli have not been found except in these lesions.

Lymphatic Glands.

In a cervical gland of Monkey 93 no lesions or bacilli have been found. In a bronchial gland of Monkey 101 there are many caseous areas and in some of these bacilli are very numerous. A cervical gland of Monkey 103 is extensively necrotic and contains very large numbers of bacilli.

SUMMARY.

The amount of disease is remarkably small.

(2.)

Animal Inoculated.—Monkey 109.

Monkey 109 was inoculated subcutaneously with 10 mg. of culture of H 53. D.H. (*a*), and was killed, when moribund, 32 days afterwards.

Lungs.

There is a general thickening of the alveolar walls with many small patches of early caseation. Bacilli are present in all the lesions but have not multiplied abundantly. The lesions, though not retrogressive in character, do not indicate a very severe infection.

Liver.

There are numerous breaking-down tubercles which are obviously progressive in character and contain large numbers of bacilli.

Kidneys.

There are small deposits of bacilli which have produced slight tissue disintegration, but no large or advanced lesions have been found.

Spleen.

The spleen contains large patches of breaking down tissue in which bacilli are very numerous.

SUMMARY.

The infection is more severe than in the preceding animals inoculated with this virus and shows severe incidence on the liver and spleen.

(3.)

Animal Inoculated.—Monkey 135.

Monkey 135 was inoculated subcutaneously with ·1 mg. of culture of H 53. D.H. (*a*), and was killed 209 days afterwards.

Lungs.

The greater part of the specimen is consolidated and shows patchy caseation with a marked tendency to fibroid change. Bacilli are very scanty.

Liver.

There are many small lesions, some of them slightly caseous. No bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

Bronchial Gland.

The gland is completely caseous. Bacilli are fairly plentiful.

SUMMARY.

A chronic type of tuberculosis with extensive fibro-caseous disease of the lungs.

VIRUS—H 59. L.B.

Animal Inoculated.—Chimpanzee 5.

Chimpanzee 5 was inoculated subcutaneously with 1 mg. of culture of H 59 .L.B. (*strain*—Human Brain), and died 79 days afterwards.

Lungs.

Small caseous tubercles are present. They are of a chronic type but do not appear to be retrogressive. Bacilli are scanty.

Liver.

Many caseous tubercles are present ; the larger have a well marked fibrous periphery. Giant cells occur in most of the lesions and multinuclear leucocytes are numerous. Bacilli are scanty.

Kidneys.

A few caseous tubercles of a chronic type are present. Bacilli are scanty.

Mesenteric Gland.

The greater part of the gland is caseous. Bacilli are scanty.

SUMMARY.

Progressive tuberculosis of a chronic type ; bacilli scanty.

VIRUS—H 60. W.B.

(1.)

Animal Fed.—Monkey 81.

Monkey 81 was fed with 1 mg. of culture of H 60. W.B. (*strain*—Mesenteric Gland), and died 50 days afterwards.

Lungs.

Parts of the tissue are normal, but there are also caseous areas of considerable size which appear to be progressive and contain fairly numerous bacilli.

Liver.

The tissue contains several discrete tubercles which are in an advanced stage of caseation. They show very little fibrous tissue formation at their margins but have not the ramifying outline found in more rapidly progressive lesions. Bacilli are scanty.

Kidneys.

In the specimen examined a few bacilli are present, but no definite lesions have been formed.

Cervical Gland.

The gland is caseous throughout and contains numerous bacilli.

SUMMARY.

Disseminated tuberculosis, apparently progressive, but relatively mild in type.

(2.)

Animal Fed.—Monkey 83.

Monkey 83 was fed with 1 mg. of culture of H 60. W.B. (*strain*—Mesenteric Gland), and died 56 days afterwards.

Lungs.

The lungs contain large, ramifying patches of consolidated tissue which are in an advanced stage of caseation. Bacilli are abundant, and the infection is obviously of a progressive type.

Liver.

Progressive tubercles of various sizes, but all containing large numbers of bacilli, are scattered abundantly throughout the tissue. The larger tubercles are in an advanced stage of caseation.

Kidneys.

Bacilli are scattered about in small numbers and have produced early lesions.

SUMMARY.

Acute disseminated tuberculosis.

VIRUS—H 61. E.C.

Animal Inoculated.—Monkey 77.

Monkey 77 was inoculated subcutaneously with 1 mg. of culture of H 61. E.C. (*strain*—Human Brain), and died 113 days afterwards.

Lungs.

The tissue is almost completely consolidated and is very extensively caseous. Bacilli are very numerous.

Liver.

Miliary tubercles, of various sizes, are thickly scattered throughout the tissue. Some of the larger are crowded with bacilli, but in the smaller bacilli are not very numerous. Bacilli are often found within parenchymatous liver cells apart from definite tubercles.

Kidneys.

Tubercles, both large and small and in various stages of degeneration, are present. Bacilli are very numerous.

Axillary Gland.

The gland is almost completely caseous and is swarming with bacilli.

SUMMARY.

Advanced infection, of an acute type, with large numbers of bacilli.

VIRUS—H 71. L.V.

Animal Inoculated—Monkey 195.

Monkey 195 was inoculated subcutaneously with 1 mg. of culture of H 71. L.V. (*b*), and died 36 days afterwards.

Lungs.

The tissue is beset with miliary tubercles which are of an acute type and contain numerous bacilli.

Liver.

The liver contains many progressive, caseating tubercles of various sizes. Bacilli are moderately numerous.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The spleen is in the same condition as the liver.

Bronchial Gland.

Several early, progressive tubercles are present. Bacilli are rather scanty.

SUMMARY.

Disseminated tuberculosis of a severe type.

VIRUS—H 74. D.S.

(1.)

Animal Inoculated.—Monkey 85.

Monkey 85 was inoculated subcutaneously with ·001 mg. of culture of H 74. D.S., and died 54 days afterwards.

Lungs.

The tissue is not completely airless but contains large caseous patches of consolidation which are evidently progressive. Bacilli are fairly numerous.

Liver.

Tubercles, both large and small, are numerous. The larger are extensively caseous. The lesions have no definite boundary zone but are evidently progressive. Bacilli are fairly numerous.

Kidneys.

Large, progressive, caseating tubercles are present and contain moderately numerous bacilli.

Spleen.

Many caseous tubercles, containing fairly large numbers of bacilli, are present. The disease appears to be more advanced than in the liver or kidneys.

Mesenteric Gland.

The gland is partially caseous. Bacilli are moderately numerous.

SUMMARY.

Advanced, disseminated tuberculosis. Bacilli moderately numerous.

(2.)

Animal Inoculated.—Monkey 89.

Monkey 89 was inoculated subcutaneously with 1 mg. of culture of H 74. D.S., and died 35 days afterwards.

Lungs.

Small, early lesions are present. They have no definite boundary zone. Bacilli are scanty.

Liver.

Miliary tubercles are abundant. They are infiltrated with leucocytes and are not surrounded by any peripheral zone of cells. Bacilli are numerous.

Kidneys.

Immediately beneath the capsule there is a wedge-shaped, caseous tubercle containing large numbers of bacilli. Less advanced lesions, apparently interstitial in origin and containing fewer bacilli, are present in the interior of the cortical substance.

Spleen.

The gland is very extensively caseous and contains very large numbers of bacilli.

Axillary Gland.

The gland is caseous throughout and contains enormous numbers of bacilli.

SUMMARY.

Disseminated tuberculosis, with particularly severe incidence on the spleen.

(3.)

Animal Inoculated.—Monkey 91.

Monkey 91 was inoculated subcutaneously with 10 mg. of culture of H 74. D.S., and died 30 days afterwards.

Lungs.

The tissue is almost completely consolidated and there are several caseous areas. In the caseous areas there are large masses of tubercle bacilli; in other parts of the tissue bacilli only occur in scanty numbers.

Liver and Spleen.

The liver and spleen are permeated with necrotic areas of irregular size and shape. Bacilli are present in all parts of the tissue, including the blood-vessels, and are very abundant in the larger necrotic areas. There is very little indication of tubercle formation; most of the lesions exhibit simple necrosis without any tissue reaction.

Kidneys.

Discrete, caseous tubercles are present and are partially surrounded by lymphocytes, leucocytes, and plasma cells. These tubercles contain large numbers of bacilli. Small groups of bacilli are also present in parts of the section which show no tissue change, both within glomeruli and amongst the interstitial tissue.

Portal Gland.

The gland is extensively caseous and contains large masses of bacilli.

SUMMARY.

Consolidation of the lungs; diffuse necrosis of the liver; caseous tubercles in the kidneys; bacilli very abundant everywhere.

VIRUS—H 81. P.W.

(1.)

Animal Inoculated.—Chimpanzee 7.

Chimpanzee 7 was inoculated subcutaneously with a very small dose of culture (estimated by the method of dilution to be equivalent to ·00001 mg.) of H 81. P.W., and died 92 days afterwards.

Lungs.

The lung tissue is congested and shows an infiltration of both lymphocytes and leucocytes, but there are no caseous areas and no patches definitely suggestive of tuberculosis. A few tubercle bacilli have been found.

Liver.

The specimen contains a fairly large lesion which is in an advanced stage of caseation. No tubercle bacilli have been found.

Kidneys.

One small, suspicious focus has been found, but no tubercle bacilli.

Spleen.

There are several caseous patches and some giant cells. Bacilli are present but rare.

SUMMARY.

Slight disseminated tuberculosis.

(2.)

Animals Inoculated.—Monkeys 95, 97, and 99.

Monkeys 95, 97, and 99 were inoculated subcutaneously with culture of H 81. P.W., the first receiving 1 mg., the second ·001 mg., the third a very small dose, estimated by the method of dilution as equivalent to ·00001 mg. The first died in 45 days, the second in 48 days, the third in 49 days.

Lungs.

The lungs of all three animals are infected with equal severity, and show extensive, obviously progressive patches of caseation in which bacilli are very numerous.

Livers.

The three livers are acutely infected and contain large numbers of bacilli.

Kidneys

A few lesions, containing bacilli in scanty numbers, have been found in Monkeys 95 and 99, but the specimen of Monkey 97 is normal.

Spleens.

The spleens of all three animals show advanced necrotic change and contain bacilli in very large numbers.

SUMMARY.

Acute, disseminated tuberculosis, the smallest dose having proved as efficacious as the largest.

VIRUS—H 84. M.S.

(1.)

Animal Inoculated.—Monkey 143.

Monkey 143 was inoculated subcutaneously with 1 mg. of culture of H 84. M.S., and died 110 days afterwards.

Lungs.

The greater portion of the tissue examined shows slight congestion, but is otherwise normal. Here and there small, semi-caseous tubercles are present. These are crowded with leucocytes, contain numerous tubercle bacilli, and show no sign of conservative change.

Liver.

There are many small, caseous tubercles which show no sign of conservative change, but contain bacilli in fairly large numbers.

Kidneys.

Small tubercles are present, and some of these contain numerous bacilli. The tubercles show well marked peripheral zones of leucocytes, lymphocytes, and plasma cells.

Spleen.

The spleen shows large, diffuse, semi-confluent areas of caseation and contains enormous numbers of bacilli, forming large red patches as seen with a low power.

SUMMARY.

General, progressive dissemination, with particularly severe incidence on the spleen.

(2.)

Animal Inoculated.—Monkey 231.

Monkey 231 was inoculated subcutaneously with 1 mg. of culture of H 84. M.S., and died 31 days afterwards.

Lungs.

Discrete but large and progressive caseous tubercles are present. They contain very large numbers of bacilli.

Liver.

The lesions are of the same type as in the lungs but more numerous. In some of the tubercles bacilli are very abundant.

Kidneys.

No definite tubercles have been found, but a few bacilli are present in the interstitial tissues.

Spleen.

The spleen is permeated with large caseous areas containing numerous bacilli.

Omental Gland.

The gland is almost completely caseous and contains very numerous bacilli.

SUMMARY.

Severe disseminated tuberculosis, with abundant multiplication of bacilli.

VIRUS—H 85. H.B.

(1.)

Animal Fed.—Chimpanzee 11.

Chimpanzee 11 was fed with 1 mg. of culture of H 85. H.B., and was killed 271 days afterwards.

Lungs.

There are a few small foci filled up with lymphocytes, but no definite tubercles. No bacilli have been found.

Liver and Kidneys.

Neither tubercles nor bacilli have been found.

Spleen.

Several caseous tubercles are present, but no bacilli have been found in them.

Mesenteric Gland.

The gland is extensively caseous. Bacilli are very rare.

SUMMARY.

There has been some dissemination, notably in the spleen, but the infection is of a mild type and bacilli are very scanty.

(2.)

Animal Inoculated.—Monkey 119.

Monkey 119 was inoculated subcutaneously with ·01 mg. of culture of H 85. H.B., and died 271 days afterwards.

Lungs.

The tissue is semi-solid and contains large progressive areas of caseation in which bacilli are very abundant.

Liver.

The liver contains many small caseous foci which show no definite boundary zone and are obviously progressive. Bacilli are scanty.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The tissue is almost completely caseous and contains bacilli in large numbers.

Bronchial Gland.

The gland contains large progressive areas of caseation. Bacilli are numerous.

SUMMARY.

Disseminated tuberculosis of a very severe type, with abundant multiplication of bacilli.

(3.)

Animal Inoculated.—Monkey 121.

Monkey 121 was inoculated subcutaneously with 0·1 mg. of culture of H 85. H.B., and was killed 97 days afterwards.

Lungs.

The specimen contains large solidified areas which are commencing to caseate. The consolidated patches have no boundary zone but appear to be becoming confluent. Bacilli sometimes occur in small groups, but on the whole are rather scanty.

Liver.

There are numerous minute, semi-caseous tubercles with no definite boundary zone. No bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are a few minute caseous patches, but no bacilli have been found.

Portal Gland.

There are many small, slightly-caseous patches. Bacilli are rare.

Bronchial Gland.

Similar to the portal gland.

SUMMARY

Disseminated tuberculosis. In the lungs, which are more extensively affected than other organs, the disease appears to be slowly progressive ; elsewhere it is of a mild and retrogressive type. Bacilli are scanty.

(4.)

Animal Inoculated.—Monkey 123.

Monkey 123 was inoculated subcutaneously with 1 mg. of culture of H 85. H.B., and died 132 days afterwards.

Lungs.

There are numerous partially caseous tubercles ; but these, though of irregular outline, due to their progression along the alveolar walls, have a fairly well defined cellular infiltration at their periphery and do not contain many bacilli.

Liver.

Fairly large, caseous, and apparently quiescent foci are present. No bacilli have been found in them.

Kidneys.

There are caseous lesions showing a tendency to fibroid change and evidently of a chronic type. No bacilli have been found.

Spleen.

The spleen shows extensive caseous lesions in which bacilli are rare.

Axillary Gland.

The gland is completely caseous and contains large numbers of bacilli.

SUMMARY.

Disseminated, caseous lesions, most of which contain few bacilli and, though fairly extensive, are not actively progressive.

(5.)

Animal Fed.—Monkey 125.

Monkey 125 was fed with 1 mg. of culture of H 85. H.B., and died 116 days afterwards.

Lungs.

The lungs contain large foci which are almost completely caseous, but are limited by a well-marked zone which contains numerous lymphocytes and leucocytes and a smaller number of fibroblasts. Bacilli are scanty.

Liver.

Small, apparently quiescent foci are present, which are overrun with leucocytes and lymphocytes and contain very few bacilli.

Kidneys.

No lesions or bacilli have been found.

Bronchial Gland.

There are extensive, partially caseous foci; but these are chronic in type and contain very few bacilli.

SUMMARY.

Disseminated tuberculosis of a relatively mild type; many of the lesions are large, caseous, and not definitely retrogressive; but few bacilli are present.

(6.)

Animal Inoculated.—Monkey 153.

Monkey 153 was inoculated subcutaneously with 10 mg. of culture of H 85. H.B., and was killed 59 days afterwards.

Lungs.

The lungs contain lesions which are small and not plentiful, but show no evidence of retrogression and contain numerous bacilli.

Liver.

The liver contains many small, partially caseous foci in which bacilli are present, but not numerous. The lesions are not acute in type but are not definitely retrogressive.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Small, slightly caseous foci are present, in which bacilli are rare.

Axillary Gland.

The gland contains large, caseous patches in which bacilli are very abundant.

SUMMARY.

Disseminated tuberculosis, presenting lesions which are not acute but are not definitely retrogressive.

VIRUS—H 91. H.S.

Animal Inoculated.—Monkey 133.

Monkey 133 was inoculated subcutaneously with 0·1 mg. of culture of H 91. H.S., and was killed 203 days afterwards.

Lungs.

Neither definite tubercles nor bacilli have been found.

Liver.

A few small, slightly caseous tubercles are present. No bacilli have been found in them.

Kidneys.

In the specimen examined there is a large, circumscribed, caseous tubercle in which bacilli are scanty.

Spleen and a Bronchial Gland.

No lesions or bacilli have been found.

SUMMARY.

There is some evidence of dissemination in the liver and kidneys, but the amount of disease on the whole is small, and bacilli are very scanty.

VIRUS—H 92. D.N.

Animal Inoculated.—Monkey 177.

Monkey 177 was inoculated subcutaneously with 1 mg. of culture of H 92. D.N., and died 39 days afterwards.

Lungs.

The tissue changes are typical of acute tubercular pneumonia. A special feature of the specimen is the abundant multiplication of bacilli, which are massed together in groups and colonies throughout the section.

Liver.

There are many tubercles which are almost completely caseous, show no demarcation zone, and contain very large numbers of bacilli.

Kidneys.

There are many small tubercles, acute in type and containing very numerous bacilli.

Spleen and a Lymphatic Gland.

These tissues show extensive and diffuse caseous necrotic change, and contain very large numbers of bacilli.

SUMMARY.

Acute, disseminated tuberculosis, with very abundant multiplication of bacilli.

VIRUS—H 99. L.K.

Animal Inoculated.—Monkey 151.

Monkey 151 was inoculated subcutaneously with ·01 mg. of culture of H 99. L.K., and died 36 days afterwards.

Lungs.

The lungs show progressive, caseous tuberculosis of an acute type, and contain very large numbers of bacilli.

Liver.

The liver contains many large, caseating, progressive lesions in which bacilli are very numerous.

Kidneys.

The kidneys contain large, caseous nodules in which bacilli are abundant.

Spleen and a Bronchial Gland.

Both organs are almost entirely caseous and contain very large numbers of bacilli.

SUMMARY.

Acute disseminated tuberculosis.

VIRUS—H 100. R.S.

Animal Inoculated.—Monkey 163.

Monkey 163 was inoculated subcutaneously with 1 mg. of culture of H 100. R.S. (original material), and died 86 days afterwards.

Lungs.

The tissue is beset with partially caseating foci which do not appear to be actively progressive. Bacilli are very scanty.

Liver.

A few small, slightly caseous foci are present. Only one bacillus has been found.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Large, discrete, and completely caseous tubercles are numerous. Bacilli are scanty.

Bronchial Gland.

There are some small patches of slight caseation. Bacilli are very scanty.

SUMMARY.

Abundant caseous foci in the lungs and spleen. Bacilli very scanty.

VIRUS—H 101. E.G.

Animal Inoculated.—Monkey 211.

Monkey 211 was inoculated subcutaneously with 1 mg. of culture of H 101. E.G., and died 51 days afterwards.

Lungs.

The tissue is semi-solid and is typical of acute progressive tuberculosis. Bacilli are numerous.

Liver.

The tissue is permeated with caseous, progressive bercles containing numerous bacilli.

Kidneys.

There are discrete, caseous tubercles containing many bacilli.

Spleen.

The tissue is largely replaced by caseous material containing numerous bacilli.

Bronchial Gland.

The gland shows progressive areas of caseation and contains numerous bacilli.

SUMMARY.

Acute disseminated tuberculosis.

VIRUS—H 102. N.H.

Animal Inoculated.—Monkey 213.

Monkey 213 was inoculated subcutaneously with 1 mg. of culture of H 102. N.H., and died 156 days afterwards.

Lungs.

The specimen shows large caseous tubercles with a well-marked fibrous periphery. Bacilli are very scanty.

Liver.

Large caseous tubercles are present. They are discrete and bounded by fibroblasts. No bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The lesions are similar to those in the liver. No bacilli have been found.

SUMMARY.

Disseminated tuberculosis of a chronic type. Bacilli rare.

VIRUS—H 103. N.S.

(1.)

Animal Inoculated.—Monkey 171.

Monkey 171 was inoculated subcutaneously with 1 mg. of culture of H 103. W.S., and died 111 days afterwards.

Lungs.

The tissue is consolidated, necrotic, and swarming with tubercle bacilli.

Liver.

The liver is permeated with small, caseating, progressive foci, in which bacilli are rather scanty.

Kidneys.

There are many tubercles in an early stage of caseation. Bacilli are fairly numerous.

Spleen.

The spleen contains large caseous areas in which bacilli are very abundant.

SUMMARY.

Disseminated tuberculosis of an acute type, with abundant multiplication of bacilli.

(2.)

Animal Inoculated.—Monkey 217.

Monkey 217 was inoculated subcutaneously with 1 mg. of culture of H 103. N.S., and died 102 days afterwards.

Lungs.

The lungs contain many small, discrete, and slightly caseous lesions. Though bacilli are rather scanty, the lesions present no histological evidence of retrogression.

Liver.

The liver contains many small caseous tubercles containing few bacilli but showing no demarcation zone.

Kidneys.

There are small caseous foci containing a few bacilli.

Spleen.

Bacilli are irregularly distributed and in a few places are numerous. They have produced slight caseation.

Bronchial Gland.

The gland contains large patches of complete caseation in which bacilli are fairly numerous. In the areas of breaking down cells adjacent to the caseous foci, bacilli are extremely numerous.

SUMMARY.

Disseminated, progressive infection. Bacilli not numerous in lungs, liver, and kidneys, but multiplying freely in a bronchial gland and the spleen.

VIRUS—H 107. H.H.

Animal Inoculated.—Monkey 251.

Monkey 251 was inoculated subcutaneously with 1 mg. of culture of H 107. H.H. (original material), and died 50 days afterwards.

Lungs.

The tissue is permeated with caseating tubercles of various sizes. The lesions are markedly progressive and contain numerous bacilli.

Liver.

Small and apparently progressive tubercles are numerous. Bacilli are rather scanty.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The tissue is extensively caseous and contains large numbers of bacilli.

SUMMARY.

Disseminated tuberculosis of an acute type.

VIRUS—H 108. H.R.

Animal Inoculated.—Monkey 165.

Monkey 165 was inoculated subcutaneously with 1 mg. of culture of H 108. H.R. (original material), and died 96 days afterwards.

Lungs.

The tissue is crowded with caseating semi confluent tubercles. Bacilli are abundant, and in some of the older foci have grown into dense groups and colonies.

Liver.

The tissue is permeated with progressive caseating tubercles of various sizes. Bacilli are numerous.

Kidneys.

There are numerous caseous tubercles containing dense masses of bacilli.

Spleen.

The spleen contains large, discrete, caseous tubercles which are crowded with bacilli. Bacilli are also scattered throughout the rest of the tissue and occur, in small numbers, where there is no evidence of histological change.

Bronchial Gland.

The gland contains several irregular, caseous patches in which bacilli are abundant.

SUMMARY.

Acute disseminated tuberculosis, with very abundant multiplication of bacilli.

VIRUS—H 109. M.W.

(1.)

Animal Inoculated.—Monkey 185.

Monkey 185 was inoculated subcutaneously with 0·1 mg. of H 109. M.W., and died 80 days afterwards.

Lungs.

There are a few small caseous tubercles, not manifestly progressive. Bacilli are rare.

Liver.

There are a few small, slightly caseous tubercles in which bacilli are very rare.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The tissue is almost entirely replaced by large, completely caseous tubercles which are closely set together, though each tubercle has a definite fibrous periphery. Bacilli are fairly numerous.

Bronchial Gland.

The gland contains caseous tubercles in which bacilli are scanty.

SUMMARY.

Slight evidence of dissemination in the lungs and liver; the spleen is much more severely affected.

(2.)

Animal Inoculated.—Monkey 197.

Monkey 197 was inoculated subcutaneously with 1 mg. of culture of H 109. M.W., and died 30 days afterwards.

Lungs.

The section shows progressive tubercles which are slightly caseous and are advancing along the thickened alveolar walls. Bacilli are very numerous.

Liver.

Acute miliary tuberculosis, with numerous bacilli.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The spleen is in an early stage of diffuse caseation and contains very large numbers of bacilli.

Inguinal Gland.

There are many foci of early caseation in which bacilli are abundant.

SUMMARY.

Acute disseminated tuberculosis. Abundant multiplication of bacilli.

VIRUS—H 110. J.B.

(1.)

Animal Inoculated.—Monkey 179.

Monkey 179 was inoculated subcutaneously with 0·01 mg. of culture of H 110. J.B. (*a*), and died 53 days afterwards.

Lungs.

The section shows irregular thickening of the alveolar walls and numerous tubercles, the larger being in an advanced stage of caseation and the smaller only slightly caseous. Giant cells and fibroblasts occur in the lesions. Bacilli are readily found but are not very numerous.

Liver.

The liver is densely permeated with large, progressive, caseous tubercles in which bacilli are numerous.

Kidneys.

Very extensive and progressive caseating tubercles are present. Bacilli are moderately numerous.

Spleen.

The specimen is almost completely caseous and contains numerous bacilli.

SUMMARY.

Severe, disseminated tuberculosis.

(2.)

Animal Inoculated.—Monkey 181.

Monkey 181 was inoculated subcutaneously with 0·1 mg. of culture of H 110. J.B. (*a*), and died 26 days afterwards.

Lungs.

The greater part of the tissue is consolidated and shows patches of early caseation. Bacilli are fairly numerous.

Liver.

The liver is permeated with discrete but caseous and progressive miliary tubercles which contain numerous bacilli.

Kidneys.

Small caseous tubercles are present and contain fairly numerous bacilli.

Spleen.

The tissue is very extensively caseous and contains numerous bacilli

SUMMARY.

Severe, disseminated tuberculosis.

(3.)

Animal Inoculated.—Monkey 183.

Monkey 183 was inoculated subcutaneously with 1 mg. of culture of H. 110 J.B. (*a*), and died 30 days afterwards.

Lungs.

The lungs are congested and contain small, caseating tubercles in which bacilli are very abundant. Bacilli also occur, in relatively small numbers, in other parts of the tissue.

Liver.

The liver is riddled with acute, miliary tubercles in which bacilli are abundant.

Kidneys.

There are several progressive, caseous tubercles, containing large numbers of bacilli.

Spleen and a Bronchial Gland.

The spleen and a bronchial gland show diffuse caseation with very large numbers of bacilli.

SUMMARY.

Acute, disseminated tuberculosis.

VIRUS—H 111. S.E.

Animal Inoculated.—Monkey 189.

Monkey 189 was inoculated subcutaneously with 1 mg. of culture of H 111. S.E., and died 62 days afterwards.

Lungs.

There is a diffuse thickening of the alveolar walls, with many patches of caseation in which the bacilli have multiplied abundantly.

Liver.

The liver is permeated with caseating foci of various sizes. Bacilli are abundant, and in the larger lesions have grown together into dense masses.

Kidneys.

There are several caseous tubercles in which the bacilli form dense masses.

Spleen.

The condition of this organ is identical with that of the liver.

Bronchial Gland.

The gland examined shows caseating areas with enormous multiplication of bacilli.

SUMMARY.

Acute disseminated tuberculosis with very abundant multiplication of bacilli.

VIRUS—H 112. B.B.

Animal Inoculated.—Monkey 201.

Monkey 201 was inoculated subcutaneously with 1 mg. of culture of H 112. B.B., and died 48 days afterwards.

Lungs.

There is a patchy consolidation with numerous areas of caseation. The lesions are evidently progressive. Bacilli are fairly plentiful.

Liver.

The liver is riddled with minute lesions, many of which are commencing to caseate. Bacilli are scanty.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The spleen is very extensively caseous. Bacilli are numerous.

Bronchial Gland.

There are large, progressive patches of caseation in which bacilli are numerous.

SUMMARY.

General dissemination, progressive and moderately acute in type.

VIRUS—H 114. A.U.

(1.)

Animal Inoculated.—Monkey 223.

Monkey 223 was inoculated subcutaneously with 1 mg. of culture of H 114. A.U., and died 109 days afterwards.

Lungs.

The tissue shows large progressive and semi-confluent caseous tubercles in which bacilli are plentiful.

Liver.

Caseous tubercles of various sizes occur throughout the tissue and contain large numbers of bacilli.

Kidneys.

There are discrete, caseous foci in which bacilli are numerous.

Spleen.

The spleen shows large and progressive caseous foci in which bacilli are abundant.

Bronchial Gland.

Large caseous patches are present, but bacilli are not numerous.

SUMMARY.

Disseminated tuberculosis of a progressive and moderately acute type.

(2.)

Animal Inoculated.—Monkey 225

Monkey 225 was inoculated subcutaneously with 1 mg. of culture of H 114 A.U., and died 100 days afterwards.

Lungs.

There is some thickening of the alveolar walls, but neither tubercles nor bacilli have been found.

Liver.

There are several small tubercles of a quiescent or retrogressive character. No bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The spleen shows very extensive and ramifying caseous tracts. Bacilli are scanty.

SUMMARY.

There is evidence of dissemination, with particularly severe incidence on the spleen. Bacilli are scanty and the infection is not of an acute type.

General Results.

Monkeys 67, 69, and 71.—These animals, inoculated subcutaneously with small doses (·01 to 1 mg.) of culture of H 8. S.C., have all yielded disseminated tuberculosis of a chronic type. The lesions contain few bacilli, but appear to be slowly progressive. These results are less severe than those produced in monkeys inoculated subcutaneously with the same doses of bovine bacilli.

Monkeys 93, 101, and 103.—In these animals, inoculated subcutaneously with from 1 to 10 mg. of H 53. D.H. (*a*), the amount of disease produced is remarkably small.

Monkeys 109 and 135.—These animals were also inoculated subcutaneously with H 53. D.H. (*a*). The former of the two shows more severe disease than the three preceding animals; and after the comparatively small dose of 1 mg. Monkey 135 shows extensive fibro-caseous disease of the lungs.

Chimpanzee 5, inoculated subcutaneously with 1 mg. of H. 59. L.B., a virus highly virulent for the bovine, shows a chronic, slowly progressive type of disease, with scanty numbers of bacilli. This result may be contrasted with the severe disease produced in Chimpanzee 24, inoculated subcutaneously with "·00001 mg." of a bovine virus.

Monkeys 81 and 83.—In each of these animals disseminated disease was produced after feeding with 1 mg. of H 60. W.B. (*strain*—Mesenteric Gland) and in the latter the disease was decidely acute in type.

Monkey 77, inoculated subcutaneously with 1 mg. of H 61. E.C., a virus of low virulence for the bovine, shows advanced infection of an acute type, with large numbers of bacilli. Similar results, recorded in my previous report, were obtained with ·1 and ·01 mg. of this virus.

Monkey 195.—Severe generalised tuberculosis was produced after subcutaneous inoculation with 1 mg. of H 71. L.V.

Monkeys 85, 89, and 91.—Severe disease was produced in these three animals. They were inoculated subcutaneously with ·001, 1, and 10 mg. of H 74. D.S., a virus of low virulence for the bovine.

Monkeys 95, 97, and 99.—In these animals severe disseminated disease was produced after subcutaneous inoculation with H 81. P.W.

Monkeys 143 and 231.—In these animals severe progressive disease was produced by subcutaneous inoculation with H 84. M.S.

Chimpanzee 11 and Monkey 125.—Disseminated disease of a mild type has been produced after feeding with 1 mg. of H 85. H.B.

Monkeys 119, 121, 123, and 153.—These animals were inoculated subcutaneously with H 85. H.B. In the first, ·01 mg. produced severe disseminated disease; in the three following, disseminated disease of a milder type was produced by doses, respectively, of ·1 mg., 1 mg., and 10 mg.

Monkey 133.—Slight dissemination was produced by ·1 mg., subcutaneously, of H 91. H.S.

Monkey 177.—Severe disseminated disease followed the subcutaneous inoculation of 1 mg. of H 92. D.N.

Monkey 151.—Acute disseminated tuberculosis followed the subcutaneous inoculation of ·01 mg. of H 99. L.K.

Monkey 163.—Disseminated, caseous lesions, not rich in bacilli, were produced by 1 mg., subcutaneously, of H 100. R.S. (culture from original material).

Monkey 211.—Acute, disseminated tuberculosis after 1 mg., subcutaneously, of H 101. E.G.

Monkey 213.—Disseminated tuberculosis of a chronic type after 1 mg., subcutaneously, of H 102. N.H.

Monkeys 171 and 217.—In each case disseminated tuberculosis was produced after 1 mg., subcutaneously, of H. 103. W.S. The former animal was more acutely infected than the latter.

Monkeys 251 and 165.—Acute infection after 1 mg., subcutaneously, of H 107. H.H. (original material) and 1 mg. of H 108. H.R. (original material).

Monkeys 185 and 197.—In the former animal ·1 mg. of H 109. M.W., subcutaneously, produced slight disease in the lungs and liver but severe disease in the spleen. In Monkey 197 1 mg. of the same virus produced severe disseminated tuberculosis.

Monkeys 179, 181, and 183.—In these animals, inoculated subcutaneously with H 110. J.B., severe disseminated disease has been produced.

Monkey 189.—Acute disseminated disease after 1 mg., subcutaneously, of H 111. S.E.

Monkeys 201, 223, and 225.—Disseminated disease, of a moderately severe type, after subcutaneous inoculation with H 112. B.B. and H. 114. A.U.

IV.—LESIONS IN SWINE.

I.—*Feeding with Bacilli of Low Virulence for the Bovine.*

H 50. P.H.: Pig 55.—Pig 55 was fed with 50 mg. of culture and was killed 92 days afterwards. In the specimen of lung a few small consolidated foci have been found; they have not the structure of definite tubercles but one or two tubercle bacilli have been found in them.

H 56. F.T.: Pig 37.—Pig 37 was fed with 50 mg. of culture and was killed 91 days afterwards. In the specimen of lung there are a few foci which are filled with lymphocytes and are possibly tubercular in origin; no bacilli have been found in them. No lesions or bacilli have been found in the liver. In a mesenteric gland there is a large caseous nodule in which no bacilli have been found.

H 67. E.P.: Pigs 125 and 127.—Both animals were fed with emulsions of tuberculous tissue rich in bacilli. The former was killed 7 days and the latter 12 days after feeding. From both animals specimens of lungs, liver, kidneys, spleen, and mesenteric gland have been examined, but neither lesions nor tubercle bacilli have been found.

H 68. R.B.: Pig 75.—Pig 75 was fed with culture for 19 weeks, the total dose being 1,500 mg., and was killed 176 days after the commencement of the experiment. In the lungs there are large tubercles in which no bacilli have been found; some of the lesions are necrotic and surrounded by fibroblasts, others are filled up with lymphocytes. No lesions or bacilli have been found in the liver, kidneys, or spleen. A mesenteric gland has undergone extensive fibro-caseous change; no bacilli have been found in it.

· *H 110. J.B.: Pigs 141 and 143.*—These pigs were each fed with 100 mg. of culture. Pig 141 was killed 7 days afterwards and Pig 143 thirteen days after feeding. In the specimens of lungs and livers of both animals neither lesions nor bacilli have been found. But in both cases fairly numerous bacilli have been found in the mesenteric glands and a few have also been seen in the submaxillary lymphatic glands.

II.—*Subcutaneous Inoculation with Bacilli of Low Virulence for the Bovine.*

H 50. P.H.: Pig 49.—Pig 49 was killed 92 days after subcutaneous inoculation with 50 mg. of culture. In the lungs there are isolated, necrotic tubercles, with dense fibrous walls and containing a few bacilli. Apart from these areas the alveolar walls show some general thickening but there is no evidence that this has been caused by tubercle bacilli. In the liver, kidneys, and a thoracic gland no tubercles or tubercle bacilli have been found.

H 53. D.H. (a): Pig 89.—Pig 89 was inoculated subcutaneously with 1 mg. of culture and was killed 123 days afterwards. In the specimen of lung there are several caseous tubercles, surrounded by a dense fibrous zone and obviously of a chronic type; no bacilli have been found in them. In the liver small, densely fibrous tubercles are present; no bacilli have been found in them. No lesions or bacilli have been found in the kidneys or spleen. In a portal gland there are large caseous tubercles, surrounded by a thick fibrous wall and containing no demonstrable bacilli.

H 53. D.H. (a): Pig 121.—Pig 121 was inoculated subcutaneously with 50 mg. of culture, and died 146 days afterwards. The lungs show large irregular patches of consolidation which are partially caseous and are in every respect typical of tubercular pneumonia; the lesions contain numerous bacilli and are evidently progressive. The liver and spleen contain a few chronic tubercles in which no bacilli have been found. No lesions or bacilli have been found in the specimen of kidney. A bronchial gland is in an advanced stage of caseation and contains numerous bacilli.

H 56. F.T.: Pig 43.—Pig 43 was inoculated subcutaneously with 50 mg. of culture and was killed 91 days afterwards. In the lungs there are a few small tubercles, centrally caseous, and surrounded by a broad fibrous zone; no bacilli have been found No tubercles or bacilli have been found in the liver or kidneys. In a thoracic gland there are caseous tubercles surrounded by dense fibrous walls. No bacilli have been found in them.

H 85. H.B.: Pig 117.—This animal was killed 268 days after subcutaneous inoculation with 10 mg. of culture. The lungs show extensive consolidation with advanced fibroid change; no tubercle bacilli have been found in them. In the liver there is a large fibro-caseous lesion in which no bacilli have been found. The specimen of kidney is normal. In a thoracic gland there are advanced caseous lesions in which no bacilli have been found.

General Results.

In most of the above experiments no more than a mild, chronic, and generally retrogressive type of disease has been produced in swine by human bacilli of relatively low virulence for the bovine. The feeding experiments, as well as the subcutaneous inoculations, have produced disease in the lungs.

More severe disease has been produced in Pig 121, by the subcutaneous inoculation of 50 mg. of H 53. D.H.

V.—LESIONS IN GUINEA-PIGS.

(*a*) Infections with Less Virulent Viruses.

(1.)

VIRUS—H 68. R.B.

Animal Inoculated.—G.P. 1988.

G.P. 1988 was inoculated subcutaneously with ·1 mg. of culture of H 68. R.B., and died 79 days afterwards.

Lungs.

The greater number of the alveoli are still patent but there are several small consolidated foci; these are necrotic, contain large numbers of leucocytes, and many bacilli; but the bacilli have not grown into dense masses.

Liver.

The liver parenchyma is to a large extent replaced by diffuse areas of complete caseation. Bacilli are numerous.

Kidneys.

No lesions or bacilli have been found.

Iliac Gland.

The gland contains areas of commencing and of advanced caseation. Bacilli are fairly numerous.

SUMMARY.

Severe infection of the liver; much less extensive disease of the lungs.

(2.)

VIRUS—H 70. N.W.

Animal Inoculated.—G.P. 1976.

G.P. 1976 was inoculated intraperitoneally with ·1 mg. of culture of H 70. N.W., and died 40 days afterwards.

Lungs.

The lungs contain many patches of consolidation, consisting of semi-confluent, breaking down foci. In these there are large masses of leucocytes and nuclear débris, and bacilli are extremely abundant.

Liver.

The liver is permeated with numerous small, semi-confluent, caseous tubercles which have no definite boundary zone. Bacilli are numerous in these areas and also occur in fairly large numbers throughout the rest of the tissue, where there are innumerable small foci showing slight necrotic change but not forming definite tubercles.

Kidneys.

The kidneys contain small, slightly caseous, and breaking down lesions in which bacilli are extremely numerous. In the interstitial tissue adjacent to these areas no histological changes are observable, but there is an invasion of bacilli in considerable numbers.

A Lymphatic Gland.

The gland examined is nearly filled with necrotic, caseating material and contains enormous numbers of bacilli.

SUMMARY.

Acute disseminated infection, with abundant multiplication of bacilli in most of the lesions.

(3.)

VIRUS—H 71. L.V.

Animal Inoculated.—G.P. 1989.

G.P. 1989 was inoculated intraperitoneally with 1 mg. of culture of H 71. L.V. (*a*), and died 32 days afterwards.

Lungs.

There is a generalised thickening of the alveolar walls but about half the alveoli are still patent. The rest of the tissue is consolidated and contains large masses of leucocytes, but is not definitely caseous. In the consolidated areas bacilli are very numerous; they are also freely distributed throughout the rest of the tissue.

Liver and Spleen.

The liver and spleen contain large ramifying tracts of early caseation and many areas showing slight disintegration but without definite caseation. Bacilli are numerous in the older lesions but rather scanty in the earlier.

Kidneys.

The kidney contains many small lesions, some slightly caseous and others not yet advanced to caseation. In all these situations bacilli are fairly numerous; they are also found invading the interstitial tissue where there are no signs of histological change.

SUMMARY.

Generalised, progressive infection, with severe incidence on both lungs and liver.

(4.)

VIRUS—H 81. P.W.

Animal Inoculated.—G.P. 2340.

G.P. 2340 was inoculated intraperitoneally with ·1 mg. of culture of H 81. P.W., and died 35 days afterwards.

Lungs.

The tissue is crowded with large, breaking down, caseous, confluent lesions in which bacilli are extremely numerous.

Liver.

Throughout the tissue there are numerous small tubercles which are irregular in outline and in an early stage of caseation. The lesions are becoming confluent, but the general destruction of the liver parenchyma is much less than is usual in acute infection. Bacilli are moderately plentiful in all the lesions but are much less abundant than in the lungs and have nowhere grown into large groups.

Kidneys.

In the specimen examined no lesions have been found in the cortical portion, but the medulla contains a fairly large tubercle of irregular outline. It is breaking down and filled with nuclear débris. It contains extremely large numbers of bacilli; bacilli are also found infiltrating the adjacent interstitial tissue which shows no histological change

Spleen.

Nearly the whole of the tissue is occupied with confluent caseating tubercles, containing very large numbers of bacilli.

Bronchial Gland.

The gland is almost completely necrotic and is filled with dense masses of bacilli.

SUMMARY.

Acute, disseminated infection with severe incidence on the lungs; the liver is rather less severely affected than usual.

(5.)

VIRUS—H 81. P.W.

Animal Inoculated.—G.P. 2454.

G.P. 2454 was inoculated subcutaneously with ·00001 mg. of culture of H 81. P.W., and died 184 days afterwards.

Lungs.

The tissue is nearly completely solidified; it is slightly caseous, and is crowded with groups of bacilli. Leucocytes are very abundant in the neighbourhood of the bacilli, and contain large numbers of them.

Liver.

There are irregular, ramifying tracts of caseation, without any demarcation zone. Bacilli are moderately numerous.

Kidneys.

A caseous lesion is present, in which bacilli occur in small numbers.

Spleen.

There is a network of caseous tracts. Bacilli are numerous.

SUMMARY.

Acute disseminated tuberculosis, with particularly abundant multiplication of bacilli in the lungs.

(6.)

VIRUS—H 82. G.F.

Animal Inoculated—G.P. 2444.

G.P. 2444 was inoculated intraperitoneally with ·1 mg. of culture of H 82. G.F., and died 161 days afterwards.

Lungs.

The greater part of the tissue is solidified and contains large caseous patches. Bacilli are scanty in the caseous areas but fairly numerous in the cellular parts of the lesions; they have nowhere formed large groups.

Liver.

The tissue is permeated throughout with large ramifying areas of complete and partial caseation. Bacilli are scanty and nowhere form groups.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are large areas some of which show complete and others partial necrosis, but a considerable amount of normal tissue is left. Bacilli are scanty.

Bronchial Gland.

There are extensive slightly caseous areas, showing some tendency to diffuse fibroid change. Bacilli are scanty.

SUMMARY.

Disseminated infection, relatively chronic in type, and associated with relatively small numbers of bacilli.

(7.)

VIRUS—H 85. H.B.

Animal Inoculated.—G.P. 2575.

G.P. 2575 was inoculated subcutaneously with 1 mg. of culture of H 85. H.B., and died 140 days afterwards.

Lungs.

The lungs are semi-solid with innumerable caseous patches which contain bacilli in rather scanty numbers. Leucocytes are very abundant.

Liver.

The liver is extensively necrotic. Bacilli are scanty.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Like the liver, the spleen is extensively necrotic. Bacilli are rare.

SUMMARY.

General dissemination with extensive necrotic change. Bacilli scanty.

(*b*) Infection with Dead Bacilli.

VIRUS H 80. D.M.

Animal Inoculated.—G.P. 2976.

G.P. 2976 received an intraperitoneal inoculation with 100 mg. of dead broth culture of H 80. D.M., and died 23 days afterwards.

Lungs.

The specimen of lung is almost completely consolidated. There is no caseation or other sign of tuberculosis. No bacilli have been found.

Liver and Kidneys.

No lesions or bacilli have been found.

Spleen.

Three bacilli have been found, and there are also a few suspicious foci which do not contain bacilli.

SUMMARY.

With the exception of three bacilli found in the spleen, there is no evidence of dissemination.

General Results.

The guinea-pigs examined afford examples of the effects produced in this species of animal by human tubercle bacilli of low virulence for the bovine.

In the animal inoculated intraperitoneally with a very large dose (100 mg.) of dead bacilli, practically no dissemination was found.

In all the animals inoculated with living bacilli generalised and fatal tuberculosis was produced.

Comparing the different organs examined, the kidneys have suffered the least severely. In three out of seven cases examined, the specimens of kidney selected for microscopic examination showed neither lesions nor tubercle bacilli. In all the other organs, *viz.*, lungs, liver, lymphatic glands, and spleen, tubercle bacilli were found in every specimen examined, and destructive tissue changes were always found in association with the bacilli.

In the lungs the amount of disease varied in different cases. Tubercles were always present. In one case the area occupied by the lesions was small relatively to the normal tissue ; in the other six i was extensive, amounting to from one half to nearly the whole of the total area of the section. No constant relationship was found between the extent of disease and the number of bacilli present. In four of the severely affected lungs bacilli were very abundant ; in one case the lung was extensively caseous, but showed relatively few bacilli ; in the other two animals bacilli were moderately numerous.

The livers, with one exception, were very extensively caseous. The exceptional case, where the general destruction of the liver parenchyma was below the average, showed more extensive disease, and more abundant multiplication of bacilli, in the lungs, although the inoculation was intraperitoneal. In the liver, as in the lungs, there was no constant relationship between the extent of disease and the number of bacilli present. Bacilli were usually numerous, but in two livers, where there was extensive necrosis, they were scanty.

All the spleens examined were extensively caseous. In the two cases where bacilli were scanty in the livers, they were also scanty in the spleens.

VI.—LESIONS IN FOWLS AND PARROTS.

(*a*) Fowls.

(1.)

VIRUS—H 53. D.H.

Animal Inoculated.—Fowl 19.

Fowl 19 was inoculated intravenously with 10 mg. of culture of H 53. D.H. (*a*), and died 14 days afterwards.

Lungs.

Throughout the tissue there are numerous small groups of tubercle bacilli which have produced slight necrosis in the situations where they occur but have not led to the formation of any lesions suggestive of tubercles. The majority of the bacilli resemble serum grown bacilli and show no definite signs of multiplication.

Liver.

Bacilli are distributed throughout the liver in small groups, which occur in almost every microscopic field. Some of the bacilli are longer than those usually met with in serum cultures. The bacilli are associated with an abundant and generally distributed infiltration of leucocytes and lymphocytes, and the cells in immediate contact with the bacilli show necrotic change; but there are no lesions with the definite structure of tubercles and no giant cells.

Kidneys.

A few small groups of bacilli are present but are not associated with any lesions.

Spleen.

Small groups of bacilli are present in almost every microscopic field. Many of them are longer than serum-grown bacilli. The bacilli are often associated with areas of slight necrosis and a few imperfectly formed giant cells are present.

SUMMARY.

Bacilli are generally distributed and are particularly numerous in the spleen and liver. They have not produced tubercles, but are associated with slight necrosis. The lesions do not seem sufficient to account for the death of the animal.

(2.)

VIRUS—H 53. D.H.

Animal Inoculated.—Fowl 21.

Fowl 21 was inoculated intravenously with 50 mg. of culture of H 53. D.H. (*a*), and died 12 days afterwards.

Lungs.

There are numerous groups of bacilli which resemble serum grown bacilli, but there is no sign of tubercle formation.

Liver.

There is a general infiltration of lymphocytes and leucocytes which is particularly dense at the periphery of blood-vessels. Tubercle bacilli are numerous and are generally distributed, but have not produced lesions typical of tuberculosis.

Kidneys.

A few small groups of bacilli are present.

Spleen.

Bacilli are scattered throughout the organ in fairly large numbers, and have produced foci of slight necrosis.

SUMMARY.

Similar to Fowl 19. The larger dose does not appear to have been more severe in its effects.

(3.)

VIRUS—H 68. R.B.

Animals Inoculated.—Fowls 15 and 16.

Fowls 15 and 16 were both inoculated intravenously with culture of H 68. R.B., the former receiving 10 mg. and the latter 50 mg. They were both killed 90 days after inoculation.

Lungs.

The lungs of both animals appear to have completely escaped infection.

Livers.

The two livers are alike. Surrounding some of the blood-vessels there are groups of lymphocytes, and there are some small patches where the parenchymatous cells stain imperfectly. No tubercle bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

Spleens

There are several minute areas where the cells stain imperfectly, and in a few of these there are red particles (in carbol-fuchsin and methylene blue specimens) which are probably fragments of bacilli.

SUMMARY.

The appearance of the livers and spleens suggests that destruction of the bacilli has taken place in these organs, but no definite tubercle bacilli or lesions unmistakably attributable to them have been found in any of the organs.

(4.)

VIRUS—H 81. P.W.

Animal Inoculated.—Fowl 131.

Fowl 131 was inoculated intravenously with 40 mg. of culture of H 81. P.W., and died 51 days afterwards.

Lungs.

There is a patch of consolidation, but it is not histologically suggestive of tuberculosis and no tubercle bacilli have been found in it.

Liver, Kidneys, and Spleen.

In these organs there are a few areas of cellular infiltration apparently due to some irritant, but no definite evidence of tubercles or tubercle bacilli has been found.

SUMMARY.

No evidence of tuberculosis.

(5.)

VIRUS—H 107. H.H.

Animal Inoculated.—Fowl 143.

Fowl 143 was inoculated intravenously with 10 mg. of culture of H 107. H.H. (original material), and died 30 days afterwards.

Lungs.

There are many small, slightly caseous foci in each of which several bacilli are present. Many of the bacilli are disintegrated, and there are no signs of active multiplication.

Liver.

There are many small deposits of bacilli which have caused accumulations of lymphocytes and leucocytes but no definite tubercles.

Kidneys.

A few bacilli are present but are not associated with definite lesions.

Spleen.

There are many small deposits of bacilli which have produced slight caseation but no definite tubercles.

SUMMARY.

Bacilli are disseminated in small numbers, but show no signs of active growth, and have not produced definite tubercles.

(6.)

VIRUS—H 115. N.G.

Animal Inoculated.—Fowl 133.

Fowl 133 was inoculated intravenously with 100 mg. of dead bacilli of H 115. N.G., and was killed 40 days afterwards.

Lungs.

The tissue shows very numerous small foci of partial necrosis in each of which a few bacilli may generally be found.

Liver.

Small, pale areas of partial necrosis are very numerous throughout the tissue. Bacilli are scanty.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Bacilli occur throughout the specimen in scanty numbers and are associated with small areas of partial necrosis.

SUMMARY.

The bacilli are disseminated in small numbers and have produced slight necrotic change.

(b) Parrots.

(1.)

VIRUS—H 81. P.W.

Animal Inoculated.—Parrot 1.

Parrot 1 was inoculated intravenously with 2 mg. of culture of H 81. P.W., and died 20 days afterwards.

Lungs.

There are no lesions resembling tubercles but there are several foci showing fusions of partially disintegrated cells. In these, bacilli are numerous ; in other parts of the tissue, bacilli are found here and there but not in large numbers and have not produced any definite histological change.

Liver.

There are several places in the tissue where small groups of cells of the liver parenchyma are breaking down. These lesions exhibit no cellular boundary zone. They contain small groups of bacilli. Bacilli are also found occasionally in the blood-capillaries, where they appear to have produced no tissue change.

Kidneys.

The tissue contains some small lesions which are slightly necrotic in the centre and are surrounded by a ring of plasma cells. They contain a few bacilli. Occasionally, isolated bacilli are found in other parts of the tissue showing no histological change.

Spleen.

The tissue is crowded with small, pale, partially necrotic, confluent areas, in all of which bacilli are numerous.

SUMMARY.

General dissemination ; the spleen is severely affected, other organs only slightly.

(2.)

VIRUS—H 81. P.W.

Animal Inoculated.—Parrot 3.

Parrot 3 was inoculated intravenously with 10 mg. of culture of H 81. P.W., and died 9 days afterwards.

Lungs.

Numerous clumps of bacilli are present, but no lesions have been formed.

Liver and Kidneys.

Bacilli are scattered about the tissues in small numbers but have not produced any lesions.

Spleen.

Bacilli are rather more numerous than in the liver or kidneys and occur in all parts of the specimen. In some places they are associated with slight cellular disintegration.

SUMMARY.

The bacilli have disseminated beyond the lungs in small numbers ; the spleen is the only organ showing any sign of tissue disintegration.

(3.)

VIRUS—H 81. P.W.

Animal Inoculated.—Parrot 5.

Parrot 5 was inoculated intravenously with ·1 mg. of culture of H 81. P.W., and was killed 298 days afterwards.

Lungs.

There are many small caseous foci of a chronic type. No bacilli have been found.

Liver.

No lesions or bacilli have been found.

Kidneys.

There are a few small, typically avian tubercles which are bounded by fibroblasts, contain giant cells, and are of a definitely chronic character. No bacilli have been found.

SUMMARY.

There are chronic lesions in the lungs and kidneys, but no bacilli have been found.

(4.)

VIRUS—H 81 P.W.

Animal Inoculated.—Parrot 9.

Parrot 9 received an intramuscular inoculation of 10 mg. of culture of H 81. P.W., and was killed 269 days afterwards.

Lungs.

The greater portion of the specimen examined is normal, but discrete, centrally necrotic foci are present in which bacilli are very rare.

Liver

Small necrotic foci are present and show multinucleate cell masses at their periphery. Bacilli are rare.

Kidneys.

There are minute, slightly caseous foci, in which bacilli are rare.

Heart

In the cardiac muscle there is a small, slightly caseous lesion which contains a giant cell and a few bacilli.

SUMMARY.

Chronic, disseminated lesions in which bacilli are present but rare.

(5.)

VIRUS—H 81 P.W.

Animal Fed.—Parrot 11.

Parrot 11 was fed with 50 mg. of culture of H 81. P.W., and died 73 days afterwards.*

Lungs.

There are some small caseous patches and a few giant cells. A few small groups of bacilli have been found in the caseous lesions.

Liver and Kidneys.

No lesions or bacilli have been found.

Spleen.

Several caseous lesions occur. In one of them fragments of bacilli have been found; no other evidence of bacilli has been seen.

SUMMARY.

Caseous lesions in the lungs and spleen; bacilli rare.

General Results.

Fowls.

The two fowls which died during the first fortnight after intravenous inoculation show general dissemination of bacilli, but no definite tubercle formation, though the presence of bacilli is associated with areas of slight necrotic change. The leucocytes appear to be attracted to the bacilli.

In the two fowls killed 90 days after inoculation the bacilli have disappeared; no lesions have been found in the lungs, but the livers and spleens bear traces of infection. These two fowls appear to have had less difficulty in eliminating their bacilli (belonging to the virus H 68. R.B.) than the fowls which were inoculated with B XXVI.

In the three experiments which terminated at intermediate periods, very little tissue change has been produced and the bacilli appear to be dying out.

Parrots.

Parrots 1 and 3, inoculated intravenously, died too early to justify any general conclusions. There is general dissemination, and the spleen seems to be the organ most affected. Parrots 5 and 9, killed at a late period after intravenous and intramuscular inoculation, respectively, show chronic, disseminated lesions, but very few bacilli have survived.

The parrot which was fed shows caseous lesions in the lungs and spleen, with scanty numbers of bacilli.

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* In Parrot 7, which died 129 days after intraperitoneal inoculation with 1 mg. of the same virus, the specimens of lungs, liver, kidneys, and spleen show neither lesions nor bacilli.

VII.—LESIONS IN OTHER ANIMALS.

(*a*) A Cat.*

VIRUS—H 70. N.W.

Animal Inoculated.—Cat 25.

Cat 25 was inoculated subcutaneously with 50 mg. of culture of H 70. N.W., and died 29 days afterwards.

Lungs.

An occasional bacillus has been found within the alveolar walls, but no definite lesions have been produced.

Liver.

Small deposits of bacilli are present in all parts of the tissue. They have caused destruction of the parenchymatous cells and an infiltration of leucocytes and lymphocytes. Bacilli are present within the blood capillaries in many places where there is no histological change.

Kidneys.

There are some small patches of interstitial infiltration. No bacilli have been found.

Spleen.

Small numbers of bacilli are present, occurring sometimes singly and sometimes in small groups. They have caused slight tissue destruction but no definite tubercles.

Axillary Gland.

The gland is completely caseous and contains enormous numbers of bacilli.

SUMMARY

Minute lesions have been produced in the liver. The amount of disease is not sufficient to account for death.

(*b*) Dogs.

(1.)

VIRUS—H 79. J.N.

Animal Inoculated.—Dog 31.

Dog 31 was inoculated intraperitoneally with 50 mg. of culture of H 79. J.N., and died 31 days afterwards.

Lungs.

Bacilli are numerous in all parts of the tissue and in many places have grown into groups. They have produced general thickening of the alveolar walls, patches of consolidation, and an infiltration of leucocytes and lymphocytes. There is no caseation, and the lesions have not the structure of definite tubercles. Bacilli frequently occur within blood-vessels, being contained within multinuclear and mononuclear cells. No fibrin is present.

Liver.

Bacilli are abundant in all parts of the tissue. They have produced partial necrosis of the parenchymatous cells, swelling and increase of the endothelial cells, and an infiltration of leucocytes and lymphocytes. There is no formation of definite tubercles. Bacilli frequently occur within blood-vessels and are often contained within leucocytes. No fibrin is present.

Kidneys.

There are no definite lesions, but bacilli occur frequently both within glomeruli and in the intertubular tissue.

Spleen.

Bacilli are very abundant but have produced very little tissue destruction. They are associated with large numbers of multinuclear leucocytes.

Sternal Gland.

The gland is swarming with bacilli and is partially necrotic. There is an abundant accumulation of multinuclear leucocytes and their remains.

SUMMARY.

General dissemination and abundant multiplication of bacilli, with relatively little tissue destruction.

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* Other cats have been examined, but do not seem worth reporting *in extenso*. Cat 27 was killed 86 days after intraperitoneal inoculation with 10 mg. of H 70. N.W., and Cat 29 died 77 days after intraperitoneal inoculation with 50 mg. of the same virus; specimens of lung, liver, kidney, spleen, and a lymphatic gland of each animal show neither tubercles nor tubercle bacilli. Similar negative results have been found with Cat 41, fed with 50 mg. of the same virus and killed 108 days afterwards. Slight infection has been found in the lungs and liver of Cat 43 (kitten), which died fifteen days after intraperitoneal inoculation with 50 mg. of H 71. L.V.

(2.)

VIRUS—H 79. J.N.

Animal Inoculated.—Dog 33.

Dog 33 (pup) was inoculated intraperitoneally with 10 mg. of culture of H 79. J.N., and died 32 days afterwards.

Lungs.

Bacilli, though occurring in all parts of the tissue, are less numerous than in the lungs of Dog 31. There is a more or less general thickening of the alveolar walls and there are many consolidated foci. Some of these foci are slightly caseous and show more increase of cells of endothelial type than do the lesions in the lungs of Dog 31. Leucocytes are abundant everywhere.

Liver.

The liver is permeated with pale, semi-necrotic areas, between which strands of normal parenchyma have survived. Bacilli are abundant. The tendency to necrosis is more advanced than in the liver of Dog 31.

Kidneys.

Small, slightly caseous tubercles, containing a few bacilli, are present. No bacilli have been found within glomeruli.

Spleen.

Bacilli are very abundant in many places and have produced extensive areas of caseation.

Sternal Gland.

The gland is caseous throughout and contains very large numbers of bacilli.

SUMMARY.

General dissemination ; bacilli numerous ; more tissue destruction than in Dog 31.

(3.)

VIRUS—H 81. P.W.

Animal Inoculated.—Dog 43.

Dog 43 (pup) was inoculated intravenously with 1 mg. of culture of H 81. P.W., and died 33 days afterwards.

Lungs.

The tissue shows a patchy, non-caseous consolidation. The solidified areas consist of detached epithelium, lymphocytes, leucocytes, and large numbers of cells resembling young fibroblasts. Amongst this material bacilli are scattered in large numbers.

Liver.

Islands and strands of normal parenchyma are left, but the greater part of the tissue is occupied by a cellular infiltration, amongst which the nuclei of the disintegrated liver cells have survived. The new elements consist of lymphocytes, leucocytes, and numerous fibroblasts. There are no caseous patches and no lesions typical of tuberculosis. Throughout the cellular infiltration bacilli are distributed abundantly.

Kidneys.

There are a few small patches of interstitial cellular infiltration ; these are not caseous, but contain bacilli in small numbers.

Spleen.

Bacilli are distributed in fairly large numbers throughout the tissue ; there is no caseation or tubercle formation, but there is a tendency to fibroid change.

Portal Gland.

The gland contains enormous numbers of bacilli and shows large areas of commencing necrosis. Large numbers of bacilli are intracellular. The protoplasm of degenerating large lymphocytes is often filled with them.

SUMMARY.

General dissemination, with abundant multiplication of bacilli in the lungs, liver, and a portal gland ; no formation of typical tubercles.

(c) Horses.

(1.)

VIRUS—H 81. P.W.

Animal Inoculated.—Horse 1.

Horse 1 was inoculated subcutaneously with 50 mg. of culture of H 81. P.W., and was killed 116 days afterwards.

Neither tubercles nor tubercle bacilli have been found in the specimens of lungs, liver, kidneys, or spleen. In the left prescapular gland there are a few suspicious granules which might be fragments of bacilli, but there are no lesions suggestive of tuberculosis and no unmistakable bacilli.

SUMMARY.

The animal has completely resisted infection.

(2.)

VIRUS—H 85. H.B.

Animal Inoculated.—Horse 11.

Horse 11 was inoculated intravenously with 10 mg. of culture of H 85. H.B., and died 51 days afterwards.

Lungs.

The tissue is swarming with bacilli, which form groups and colonies visible everywhere with a low power. There is no tubercle formation or tissue destruction ; bacilli often occur within otherwise normal alveolar walls ; but amongst the larger collections of bacilli there is an infiltration of lymphocytes and leucocytes.

Liver.

Bacilli are scattered throughout the tissue in large numbers and have often grown into groups. There is no tubercle formation, but collections of lymphocytes and leucocytes are often found associated with the bacilli.

Kidneys.

Small groups of bacilli occur in many parts of the tissue but have produced no definite lesions.

Spleen.

Bacilli are numerous and generally distributed ; they have produced foci of slight caseation.

Thoracic Gland.

The gland is semi-caseous throughout and is crowded with bacilli.

SUMMARY.

General dissemination and abundant multiplication of bacilli ; very little tissue destruction except in the thoracic gland.

(3.)

VIRUS—H 92. D.N.

Animal Inoculated.—Horse 7.

Horse 7 was inoculated intravenously with 10 mg. of culture of H 92. D.N., and was killed 137 days afterwards.

Lungs.

The tissue contains numerous small, irregularly shaped lesions which contain giant cells but are not caseous and are overrun with lymphocytes and leucocytes. Bacilli are rare and the general characters of the lesions are retrogressive.

Liver, Kidneys, and Spleen.

No lesions or bacilli have been found in the specimens examined.

Bronchial Gland.

The gland contains caseous tubercles of a conservative type in which bacilli are rare.

SUMMARY.

Slight, retrogressive infection of the lungs and a bronchial gland. No lesions or bacilli found in the other organs examined.

(*d*) Goats.

(1.)

VIRUS—H 53. D.H.

Animal Inoculated.—Goat 65.

Goat 65 (a kid) was inoculated subcutaneously with 10 mg. of culture of H 53. D.H. (*a*), and died 38 days afterwards.

Lungs.

The specimen is almost completely consolidated and contains many caseous patches. Giant cells are numerous. Bacilli occur in small numbers in all parts of the specimen.

Liver.

There are several small, slightly caseous tubercles. Giant cells are numerous. No bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are several small, caseous foci in which giant cells are numerous. A few bacilli are present in each of these foci.

Mesenteric Gland.

There are numerous caseous patches containing many giant cells. Bacilli are scanty.

SUMMARY.

Disseminated tuberculosis of a chronic type.

(2.)

VIRUS—H 85. H.B.

Animal Inoculated.—Goat 63.

Goat 63 was inoculated subcutaneously with 10 mg. of culture of H 85. H.B., and was killed 402 days afterwards

Lungs.

Discrete, retrogressive, and densely calcareous tubercles are present. A few bacilli have been found in the centre of the lesions.

Liver and Kidneys.

These organs contain tubercles identical in histological characters with those found in the lungs. Bacilli are present but rare.

Spleen and Popliteal Gland.

The tissues are almost entirely replaced by calcareous material. A few bacilli have been found.

SUMMARY.

General dissemination ; lesions retrogressive and densely calcareous but still containing a few bacilli.

(3.)

VIRUS—H 98. B.R.

Animal Inoculated.—Goat 69.

Goat 69 was inoculated subcutaneously with 10 mg. of culture of H 98. B.R., and was killed 114 days afterwards.

Lungs.

There are many semi-necrotic foci in which parasitic worms (strongyli) are present ; but neither definite tubercles nor tubercle bacilli have been found.

Liver, Kidneys, and Spleen.

Neither tubercles nor bacilli have been found.

Mammary Gland.

The specimen of the left gland shows numerous tubercles. These are centrally caseous and have a dense fibrous periphery. No bacilli have been found.

The specimen of the right gland shows numerous lesions, similar on the whole to those in the left gland but with less advanced fibrous tissue formation. A few bacilli have been found.

Left Prescapular Gland.

Neither tubercles nor bacilli have been found.

SUMMARY.

The bacilli have travelled to the mammary gland and have there set up extensive tuberculosis of a chronic type. No definite evidence of tuberculosis has been found in the other organs examined.

(4.)

VIRUS—H 110. J.B.

Animal Inoculated.—Goat 79.

Goat 79 was inoculated subcutaneously with 50 mg. of culture of H 110. J.B., and was killed 9 days afterwards.

Lungs.

The tissue is congested and shows many small foci which are crowded with leucocytes and are commencing to caseate. The lesions are well vascularised. Bacilli are present in these sites but are scanty. The condition of the lungs indicates recent infection.

Liver, Kidneys, and Spleen.

Neither lesions nor bacilli have been found.

Prescapular Gland.

The gland is almost completely caseous and contains numerous bacilli.

Mammary Gland.

Neither tubercles nor bacilli have been found.

SUMMARY

The lungs show many early lesions containing a few bacilli. Bacilli are abundant in the prescapular gland.

(5.)

VIRUS—H 115. N.G.

Animal Inoculated—Goat 81.

Goat 81 was inoculated intravenously with 2 mg. of culture of H. 115. N.G., and died 41 days afterwards.

Lungs.

In one specimen the tissue is highly congested, with very little air space left ; bacilli occur in all parts of the tissue and in some places are associated with groups of broken down leucocytes, but they have not produced definite tubercles. In another specimen there is irregular thickening of the alveolar walls and some bacilli are present, but there are no tubercles.

Liver.

No definite tubercles have been found, but there are numerous patches of cellular infiltration, amongst which bacilli are occasionally found.

Kidneys and Spleen.

A few bacilli have been found, but no tubercles.

Bronchial Gland.

There are several caseous foci in which bacilli are fairly plentiful.

Udder.

Four different specimens have been examined, but neither tubercles nor bacilli have been found.

SUMMARY.

In all the organs examined except the udder bacilli have been found. They are numerous in the lungs, which are partially consolidated but do not contain typical tubercles. In the liver, kidneys, and spleen bacilli are scanty and have not formed typical tubercles.

General Results.

Cat 25.—With the exception of the axillary gland, which is completely caseous and overwhelmed with bacilli, very little histological change has been produced. There is, however, evidence of dissemination, particularly in the liver, where bacilli are found within the blood capillaries.

Dogs 31, 33, and 43.—All three animals show general dissemination and abundant multiplication of bacilli. The two first were inoculated intraperitoneally, and the last intravenously, but the three animals seem to have all succumbed to the toxic effects of the inoculation, and show an absence of typical tubercle formation. In the animal inoculated with the larger intraperitoneal dose (50 mg.), bacilli are circulating freely in the blood stream, and there is little tissue destruction ; in the dog inoculated with 10 mg. intraperitoneally, though the duration of life was the same, there is much more caseous and diffuse necrotic change ; the tissues of the animal which died, in about the same time, after 1 mg. intravenously inoculated, show little caseation, but some attempt at conservative change. Though the animals were inoculated with human viruses of relatively low virulence for the rabbit or bovine, the disease is quite as severe as that produced in the dog by bovine viruses.

Horse 1 completely resisted subcutaneous inoculation with 50 mg. of a human virus of relatively low virulence for the bovine or rabbit. The same result was obtained with Pony 6, inoculated subcutaneously with 50 mg. of a bovine virus.

Horse 11, inoculated intravenously with 10 mg. of H 85. H.B., died in 51 days, with general dissemination and abundant multiplication of bacilli but little tissue destruction.

Horse 7, inoculated intravenously with the same dose of H 92. D.N., shows very high resistance to infection.

Goats 63 and 65, inoculated subcutaneously with 10 mg. of viruses of relatively low or intermediate virulence, show disseminated tuberculosis of a chronic type.

Goat 69, also inoculated subcutaneously with 10 mg. of a virus of relatively low virulence, exhibits extensive chronic tuberculosis of the mammary gland.

Goat 79 was inoculated subcutaneously with a virus of relatively high virulence. There is evidence of dissemination in the lungs, 9 days after inoculation.

Goat 81, inoculated intravenously with 2 mg. of a virus of relatively low virulence, shows generalised dissemination of bacilli without typical tubercle formation.

C.—LESIONS PRODUCED BY MAMMALIAN TUBERCLE BACILLI ISOLATED FROM SWINE.

I.—LESIONS IN CALVES.

(*a*) Viruses of High Virulence.

Certain viruses obtained from spontaneously infected swine and found to possess the general characters of mammalian tubercle bacilli were tested upon calves by the subcutaneous inoculation of 50 mg. of culture.

Tissues from the following animals, in which fatal infection was produced in less than 90 days (with one exception), have been microscopically examined.

CALVES FATALLY INFECTED WITH 50 MG. OF A SWINE VIRUS.

Virus Inoculated.	Number of Calf.	Duration of Experiment in Days.
P V.	336	29
P VI.	326	29
P IX.	334	45
P XI.	338*	37
P XII.	376	19
P XII.	444	48
P XII.	450	41
P XIV.	446	80
P XIV.	448	104
P XIV.	532	76
P XIV.	534	87
P XIV.	536	46
P XIV.	538	32

* Dose—46 mg.

All the animals in this list were either found dead or were killed when very ill. Calf 448 lived longer than any of the others, and was killed 104 days after the commencement of the experiment.

Specimens have been examined of the lungs, liver, kidneys, and lymphatic glands of each animal. The same histological methods have been employed as in the investigations of lesions produced by bovine or human bacilli.

The lesions produced are typical of acute progressive tuberculosis, and both histologically and in respect of the distribution and characters of bacilli are identical with those produced in calves fatally infected with 50 mg. doses of bovine or human viruses. A full description of the histological details has been given in Volume IV. of the Appendix to the Second Interim Report.

It is only necessary to add here a few special details which have been noted in particular instances.

P XII.: Calf 376.—This animal died in an unusually short time, 19 days. The lungs are typical of acute infection and contain numerous bacilli, but necrosis is less advanced than in cases which have run a longer course. The liver is also typical, containing innumerable caseous miliary tubercles. No lesions have been found in the specimens of kidney. In a popliteal gland there are many small caseous lesions which are obviously progressive and contain numerous bacilli.

P XIV.: Calf 446.—This animal died in 80 days. The lungs are typical of acute tuberculosis. No lesions or tubercle bacilli have been found in the specimens of liver. The kidneys are typical of suppurative nephritis; no tubercle bacilli have been found in the specimens. A popliteal gland contains small caseous lesions which are not circumscribed; a few tubercle bacilli are present.

P XIV.: Calf 448.—This animal was killed after surviving the infection for 104 days. The lungs exhibit severe, progressive infection, but with less necrosis than is usual in very acute cases. In the liver there is a small, circumscribed tubercle containing a few bacilli, but no miliary tubercles of the acute type have been noted. In the kidneys there are several smal patches of cellular infiltration and a few giant cells, but the lesions have not become caseous; tubercle bacilli are scanty. In an axillary gland no lesions or bacilli have been found. Apart from the lungs, therefore, there is much less evidence of actively progressive disease than is usual with a virulent virus.

P XIV.: Calf 532.—The lungs of this animal, which died 76 days after inoculation, are typical of acute infection. No lesions or bacilli have been found in the specimens of liver, kidney, and a mesenteric gland.

P XIV.: Calf 534.—This animal was killed 87 days after inoculation. The lungs contain large caseous lesions which are obviously progressive but contain fewer bacilli than are usual in acute infections; earlier lesions are also present, but are less numerous and show less necrotic change than is usual in acute cases. In the specimen of liver no tubercle bacilli have been found and no definite tubercles. In the kidney there are a few patches of interstitial infiltration, which from their character appear to be due to infection by tubercle bacilli, but no bacilli have been found. In a gluteal gland there are some small areas of early caseation containing giant cells; bacilli are rather scanty.

P XIV.: Calf 536.—In all the tissues of this animal, inoculated with culture isolated from Calf 448, the lesions are typical of highly acute infection and contain numerous bacilli.

P XIV.: Calf 538.—In the lungs the lesions are definitely acute in type and contain numerous bacilli, but perhaps they are less advanced than is usual in acute infections. In the liver miliary, caseous tubercles are present and contain many bacilli but are not very numerous. In the kidneys there are numerous caseating lesions with moderately large numbers of bacilli; one of the lesions appears to have commenced within a glomerulus. In a popliteal gland there are small caseous foci containing numerous bacilli.

General Results.

With the exception of some of the animals inoculated with the virus P XIV., the lesions produced are all typical of highly acute infection, and are identical with those found in calves inoculated with similar doses of highly virulent human or bovine bacilli. In the case of P XIV. the deviations from this standard of virulence are not very great; all the calves show severe, progressive disease of the lungs, but in two out of the six the lesions in the lungs are not of the rapidly acute type, and the amount of disease in the other organs is relatively small.

(b) A Virus of Lower Virulence.

VIRUS—P IV.

(1.)

Animal Inoculated.—Calf 312.

Calf 312 was inoculated subcutaneously with 50 mg. of culture of P IV., and was killed, when apparently in good health, 90 days afterwards.

Lungs.

Three specimens of lung have been examined. (1) In the first there is some slight, irregular thickening of the alveolar walls and there are a few small consolidated foci which are chiefly occupied with lymphocytes. These foci are not caseous and are not histologically distinctive of tuberculosis ; two or three of them are found in the interlobular connective tissue. In some of these foci, including those found in the interstitial tissue, one or two tubercle bacilli are present ; no bacilli are found elsewhere. (2) In the second specimen the pleural surface is thickened and vascularised ; it contains three small nodules in which the predominant cells are lymphocytes ; the nodules are not caseous but contain an occasional tubercle bacillus. The lung parenchyma contains several small tubercles of the so-called "small cell" type. They are not caseous but show increase of the endothelial cells and are densely infiltrated with both lymphocytes and leucocytes. Each of them contains several bacilli. Plasma cells are numerous. Some of the bronchioles are filled with leucocytes and other cells, and contain numerous tubercle bacilli. (3) The third specimen is a typical example of tubercular pneumonia. It is filled with irregular, ramifying, caseous tubercles, which have a broad cellular margin containing many giant cells. Smaller tubercles, containing giant cells but not caseous, are also present. Between these areas small tracts of lung tissue which is not completely solidified are found. These are compressed, in a catarrhal condition, and show early tubercles with giant cell formation. Tubercle bacilli are very numerous ; they are particularly abundant in the caseous areas ; in these situations they are often aggregated into groups which are visible with a low power. They also form groups in some of the giant cells. The lesions are evidently progressing, and bacilli are found in some of the alveoli which have not become converted into tubercles.

Liver.

The liver contains a few minute foci which are not caseous but are overrun with lymphocytes. There are a few giant cells in these areas. Bacilli are very scanty, but a few have been found in one of the lesions

Kidneys and Spleen.

Neither tubercles nor tubercle bacilli have been found in the specimens examined.

Heart.

Immediately beneath the endocardium of the right ventricle there is a small area of cellular infiltration. The infiltrating cells are mainly lymphocytes, but there are also some multinuclear leucocytes and there is some proliferation of endothelial cells. A few newly formed blood capillaries are present, and there are some minute foci where the cell outlines are indistinct and necrosis appears to be commencing. A few tubercle bacilli are present.

Suprarenal Capsule.

The suprarenal capsule contains in the cortex many caseating tubercles which have a broad cellular margin and contain many giant cells. Tubercle bacilli are present in all the tubercles, both in the caseous areas and in the cellular margins ; and in some of the tubercles bacilli are plentiful.

Bronchial Gland.

The gland examined is nearly normal, but contains one or two minute, semi-caseous foci in which a few bacilli are present.

Portal Gland.

In a portal gland there is a small tubercle containing a large giant cell and a few bacilli.

Popliteal Gland.

No tubercles or bacilli have been found.

SUMMARY.

Slowly progressive tuberculosis of the lungs ; disseminated lesions, of a chronic type, in many of the other organs and lymphatic glands.

(2.)

Animals Inoculated.—Calves 314, 356, 358.

These animals received subcutaneous inoculations of cultures of P IV. Calf 314 received 34 mg. of the original strain, and was killed 135 days afterwards ; Calf 356 received 50 mg. of culture isolated from Calf 312, and was killed 105 days afterwards ; Calf 358 received 50 mg. of the strain from Calf 312, and was killed 93 days afterwards.

Calf 314.—No tubercles or tubercle bacilli have been found in the specimens of lung, liver, kidney, popliteal gland, mediastinal gland, mesenteric gland, and suprarenal capsule.

Calf 356.—No tubercles or bacilli have been found in the lung (2 pieces) or prescapular gland. A few suspicious foci have been noted in the kidney, but no bacilli have been found in them. In the liver there are a few small foci, of doubtful nature, infiltrated with leucocytes ; no bacilli have been found in them.

Calf 358.—No lesions or bacilli have been found in specimens of the lungs, liver, kidneys, a mediastinal gland, a portal gland, and a popliteal gland.

SUMMARY.

The absence of disease in these animals is in marked contrast to the condition of Calf 312.

(3.)

Animal Inoculated.—Calf 426.

Calf 426 received an intra-muscular inoculation with 200 mg. of culture of **P IV.** (through Calf 358), and died 29 days afterwards, apparently from diarrhœa.

Lungs.

The alveolar walls are thickened and there is a considerable degree of leucocytosis. Several small foci are present which are commencing to caseate. These foci, which are infiltrated with leucocytes, have no definite boundary zone and exhibit no definite structure. No giant cells are present. No fibrin is demonstrable. Bacilli are fairly numerous ; they are generally curved and from 3 to 4·5 μ in length.

Liver.

Like the lungs, the tissue is infiltrated with oxyphil leucocytes. Many small, slightly necrotic foci are present. These have no definite structure and no definite boundary zone, but contain numerous leucocytes and occasionally a few giant cells. No fibrin is demonstrable. Tubercle bacilli are present but scanty.

Kidneys.

The tissue shows marked interstitial fibroid change. No tubercles or tubercle bacilli have been found.

Prescapular Gland.

The gland shows extensive fibro-caseous degeneration, with a marked tendency to fibroid change. Bacilli are numerous.

Spleen.

Bacilli are distributed throughout the tissue in small groups, but have produced no appreciable tissue destruction.

SUMMARY.

Numerous small disseminated foci are present in the lungs and liver, and there is no definite evidence that these are in a retrogressive condition.

General Results.

The effects produced by the virus P IV. are irregular. Calf 312 shows slowly progressive tuberculosis of the lungs and disseminated lesions of a chronic type in many of the other organs. In Calves 314, 356, and 358 I have found no microscopic evidence of dissemination. Calf 426 shows many small tubercles, not obviously in a retrogressive condition, in the lungs and liver ; but the animal was in poor health when inoculated and died from intercurrent disease.

Two other porcine viruses, P XLI. and P XLVI., were found to be of low virulence for calves, but as they failed to produce dissemination beyond the nearest glands no useful material was available for microscopic examination.

II.—LESIONS IN RABBITS.

The viruses, possessing the characters of mammalian tubercle bacilli, which have been isolated from swine have been inoculated into rabbits by Drs. A. S. and F. Griffith.

I have made microscopic examinations of tissues obtained from the following animals :—

RABBITS INOCULATED WITH SWINE VIRUSES.

Virus Inoculated.	Dose.	Mode of Inoculation.	No. of Rabbit.	Duration of Experiments in Days.
P IV.	·1 mg.	Intravenous	307	D 86
P IV. (direct).	1 mg.	Intravenous	461	K 90
P IV. (direct).	1 mg.	Intraperitoneal	462	K 90
P IV. (direct).	10 mg.	Intraperitoneal	463	D 18
P IV. (Calf 312).	1 mg.	Intravenous	555	D 88
P IV. (Calf 312).	50 mg.	Subcutaneous	557	D 149
P XIX.	·1 mg.	Intravenous	622	D 21
P XXXIV.	·1 mg.	Subcutaneous	981	D 146
P XXXVI.	·1 mg.	Intravenous	1094	D 26
P XXXVII.	·1 mg.	Intravenous	1100	D 21
P XL.	1 mg.	Intravenous	1120	D 17
P XL.	1 mg.	Intravenous	1123	K 38
P XL.	10 mg.	Intravenous	1124	K 38
P XLIII.	·1 mg.	Intravenous	1127	D 18
P XLVI.	1 mg.	Intraperitoneal	1047	K 100
P XLVI.	10 mg.	Intraperitoneal	1048	K 100

D = Died. K = Killed.

P. IV.: Rabbit 307.—In the lungs some large caseous nodules are present and, apart from these areas, there is some thickening of the alveolar walls. In the caseous foci bacilli are present, but are not very numerous. A few suspicious foci are present in the liver, but no definite tubercles have been found, and no bacilli. Slightly caseous lesions with broad cellular margins are present in the kidneys. They contain few bacilli.

P IV. (*direct*) : *Rabbit 461.*—The lungs contain numerous caseous tubercles many of which are confluent; they contain giant cells and are poor in bacilli; around many of the foci there is a peripheral zone of lymphocytes and plasma cells, but some of the foci of commencing caseation are not marked off by a boundary zone; the lesions on the whole appear to be slowly progressive. No tubercles or bacilli have been found in the liver. In the kidney there are several large caseous tubercles which are surrounded by a broad layer of lymphocytes and plasma cells; bacilli are numerous and in some of the caseous areas have grown into large groups.

P IV. (*direct*) : *Rabbit 462.*—In the lungs there are many small tubercles which are caseous, contain giant cells and numerous oxyphil leucocytes, and are surrounded by a zone of lymphocytes and plasma cells; bacilli are scanty. No tubercles or bacilli have been found in the liver. Caseous tubercles are present in the kidneys and are identical in characters with those found in the kidneys of Rabbit 461.

P IV. (*direct*) : *Rabbit 463.*—The lungs show a general thickening of the alveolar walls and contain several small consolidated foci which show cell fusions but are not caseous; bacilli are present in the lesions but scanty. In the liver there are no large lesions but some giant cells are present, and these occasionally contain bacilli. In the kidneys no tubercles have been found, but a few bacilli occur in the interstitial tissue. In the spleen there are several slightly caseous lesions containing bacilli in small numbers.

P IV. (*Calf 312*) : *Rabbit 555.*—The lung is semi-solid and is occupied by large, confluent, caseous areas which are obviously progressive; bacilli are abundant; the lungs appear to be more severely affected than those of Rabbit 461. In the liver there are a few minute tubercles; only one bacillus has been found. The kidneys contain many caseous tubercles, some large and some small; in the larger, bacilli are very abundant; many of the bacilli are notably long; the tubercles appear to be progressive.

P IV. (*Calf 312*) : *Rabbit 557.*—The lungs contain many tubercles, both large and small, which are of a slowly progressive type. The larger tubercles are almost completely caseous; at their margin there is an irregular zone of leucocytes, lymphocytes, and some plasma cells, but external to this zone the tissue is becoming consolidated and contains tubercle bacilli in scanty numbers. Within the central portions of the tubercles bacilli are fairly numerous. The majority of the bacilli are curved and are notably long. In the specimen of liver no lesions or bacilli have been found. In the kidneys there are many caseous tubercles containing numerous bacilli both centrally and at their periphery. In the suprarenal capsule there are large caseous tubercles containing numerous bacilli.

P XIX. : *Rabbit 622.*—The lungs, liver, and spleen of this animal are typical of acute, fatal infection, and contain large numbers of bacilli. No lesions or bacilli have been found in the kidneys.

P XXXIV. : *Rabbit 981.*—The specimen of lung is almost completely consolidated; there are irregular; fairly advanced areas of caseation; tubercle bacilli are rather scanty. In the liver there are many small foci showing partial obliteration of the liver cells, infiltration with lymphocytes and leucocytes, and occasionally a tendency to giant cell formation; bacilli are scanty. In the kidneys there are patches of interstitial cellular infiltration and a few areas of slight caseation; bacilli are fairly abundant.

P XXXVI. : *Rabbit 1094.*—The lungs, liver, and a mediastinal gland show highly acute infection typical of intravenous inoculations with virulent mammalian bacilli. The kidneys are less severely infected but show many patches of infiltration containing numerous bacilli.

P XXXVII. : *Rabbit 1100.*—The lungs and liver are typical of acute infection by intravenous inoculation. The kidneys show a diffuse cellular infiltration and contain many large caseous areas; bacilli are distributed throughout the tissue in moderately large numbers.

P XL. : *Rabbit 1120.*—The lungs are almost completely solidified and are typical of acute intravenous infection; bacilli are very numerous. The liver is also acutely infected and shows diffuse early caseation; bacilli are particularly numerous. The kidneys contain numerous early foci in which bacilli are abundant.

P XL. : *Rabbit 1123.*—Two specimens of lung have been examined but no tubercles have been found; the tissue appears histologically normal, except for a few patches of slight alveolar thickening; in one of the specimens a few isolated tubercle bacilli have been found. No lesions or bacilli have been found in the specimens of liver and kidney. In the specimen of spleen there is one small caseous focus containing a few bacilli. In a portal gland there are one or two very small, slightly suspicious foci; only one bacillus has been found.

P XL. : *Rabbit 1124.*—The lungs show some areas of collapse; there is some thickening and infiltration of the alveolar walls, but no caseous foci have been found; tubercle bacilli are present but are rare. In the liver there is an infiltration with lymphocytes and leucocytes, which form numerous small groups; in some of these doubtful foci giant cells occur; tubercle bacilli are present in scanty numbers. No lesions or bacilli have been found in the kidney. In a mesenteric gland there is a caseous tubercle surrounded by fibroblasts and containing a few bacilli.

P XLIII. : *Rabbit 1127.*—The lungs, liver, and a mediastinal gland are typical of highly acute infection with rapid tissue destruction and enormous numbers of bacilli. In the kidney the lesions are much smaller and obviously more recent; bacilli are much less numerous.

P XLVI. : *Rabbit 1047.*—Two specimens of lung have been examined; both are in a semi-solid condition with an irregular distribution of caseous areas typical of tubercular pneumonia. There is a tendency for some of the areas to be bounded by a zone of lymphocytes, plasma cells, and fibroblasts. Tubercle bacilli are numerous in many of the caseous areas, and in a few have grown into conspicuous colonies. In tubercles where they are less numerous, the bacilli present are generally longer than in situations where they appear to be rapidly multiplying. In the specimen of liver neither tubercles nor bacilli have been found. In the kidney there are some patches of interstitial infiltration containing large numbers of plasma cells; no tubercle bacilli have been found.

P XLVI. : *Rabbit 1048.*—In the lung there are several small tubercles; some of these are caseous, but others are completely overrun with lymphocytes; no tubercle bacilli have been found. In the liver there is a caseous lesion surrounded by a broad fibrous zone; no bacilli have been found in it. In the kidney there is a caseous tubercle containing a few bacilli.

General Results.

Both in general characters and in minute details the lesions produced in rabbits by porcine bacilli are identical with those caused by human and bovine bacilli.

III.—LESIONS IN SWINE.

(*a*) FEEDING WITH BACILLI OF RELATIVELY LOW VIRULENCE FOR THE BOVINE.

P IV. : *Pig 124.*—Pig 124 was fed with 1 mg. of culture, and was killed 118 days afterwards. In the specimens of lungs, liver, and kidneys no lesions or bacilli have been found. A submaxillary lymphatic gland contains necrotic fibro-caseous tubercles, in which bacilli are scanty

P IV.: Pig 134.—Pig 134 was fed with 1 mg. of culture, and was killed 128 days afterwards. No tubercles or bacilli have been found in the lungs or kidneys. In the specimen of liver there is a patch of scar tissue which has replaced a portion of the liver parenchyma. It has not the structure of a tubercle. No tubercle bacilli have been found. In a submaxillary lymphatic gland there is a small caseous tubercle surrounded by a broad zone of fibrous tissue. Bacilli are scanty.

(*b*) SUBCUTANEOUS INOCULATION WITH BACILLI OF RELATIVELY LOW VIRULENCE FOR THE BOVINE.

P IV.: Pig 126.—Pig 126 was inoculated subcutaneously with 1 mg. of culture, and was killed 114 days afterwards. In the lungs several isolated, caseous tubercles are present. They are of the chronic type and are completely surrounded by fibroblasts. At the periphery of the caseous material there is a dense ring of oxyphil leucocytes. Bacilli are present in small numbers in some of the tubercles. There are a few small, suspicious foci in the liver, but no definite tubercles and no tubercle bacilli have been found. No lesions or bacilli have been found in the kidneys. In a portal gland there is a caseous tubercle surrounded by a thick wall of fibrous tissue. No bacilli have been found.

P IV.: Pig 132.—Pig 132 was inoculated subcutaneously with 1 mg. of culture, and was killed 122 days afterwards. No lesions or bacilli have been found in the lungs, liver, or kidneys. In a portal gland there are several obviously retrogressive tubercles. No bacilli have been found.

P XLI.: Pig 166.—Pig 166 was inoculated subcutaneously with 50 mg. of culture, and was killed 102 days afterwards. In the lung there is a consolidated patch, not typical of tuberculosis; a few isolated tubercle bacilli have been found in it. In the specimens of liver, kidney, spleen, and bronchial gland no lesions or bacilli have been found.

P XLI.: Pig 202.—Pig 202 was inoculated subcutaneously with 50 mg. of culture derived from Pig 166, and died 64 days afterwards. In the lungs there are large areas of consolidation which contain caseous patches; the cellular elements of the lesions show a tendency to fibrous tissue formation; bacilli are scanty, but are not restricted to the caseous areas. The liver contains several small lesions which are partially surrounded by fibroblasts and contain giant cells; bacilli are rare. In the specimen of kidney no lesions or bacilli have been found. In the spleen there are small lesions similar to those found in the liver; one bacillus has been found. In a bronchial gland there are numerous giant cells and patches of slight caseation; bacilli are scanty.

P XLI.: Pig 258.—Pig 258 was inoculated subcutaneously with 50 mg. of culture of P XLI, and died 103 days afterwards. The specimen of lung is nearly solid, and typical of caseous pneumonia; tubercle bacilli are very numerous. The liver contains many small caseous tubercles with no definite boundary zone, and also isolated giant cells; the lesions contain bacilli in small numbers. The kidneys contain a few small lesions in which bacilli are scanty. In the spleen bacilli are scattered throughout the tissue in fairly large numbers and have produced areas of partial caseation. In a precrural gland bacilli are distributed rather scantily, and have led to the formation of giant cells and areas of partial caseation.

P XLVI.: Pig 164.—Pig 164 was inoculated subcutaneously with 50 mg. of culture, and was killed 97 days afterwards. No lesions or bacilli have been found in the specimens of lung, liver, kidney, and spleen.

General Results.

The results both with feeding and by subcutaneous inoculation are similar to those produced in the comparable experiments made with human viruses of relatively low virulence for the bovine, the disease being as a rule slight in amount and retrogressive in character. But in two cases a subcutaneous dose of 50 mg. has produced severe disease of the lungs.

IV.—LESIONS IN GUINEA-PIGS.

(1.)

VIRUS—P XLI.

Animal Inoculated.—G.P. 2195.

Guinea-pig 2195 was inoculated subcutaneously with 1 mg. of culture of P XLI, and died 58 days afterwards.

Lungs.

The lungs are semi-solid and in a condition of early caseation. The lesions do not appear to be rapidly progressive; though not marked off by definite boundary zones they contain large numbers of cells resembling fibroblasts and show relatively little tissue destruction. Bacilli are scattered throughout the tissue but are only moderately abundant.

Liver and Spleen.

The liver and spleen show a diffuse caseous infiltration. Bacilli are scattered throughout the organs, but not in large numbers.

Kidneys.

No lesions or bacilli have been found.

Axillary Gland.

The gland is extensively caseous. Bacilli are fairly numerous.

SUMMARY.

The liver and spleen are extensively caseous. The lesions in the lungs are widespread, but less necrotic than in the liver and spleen. Bacilli are moderately numerous.

(2.)

VIRUS—P LIV.

Animal Inoculated.—G.P. 2571.

G.P. 2571 was inoculated subcutaneously with ·1 mg. of culture of P LIV (a porcine virus of high virulence for the rabbit), and died 54 days afterwards.

Lungs.

The lungs contain many patches of consolidation which are commencing to break down. In most of these areas some of the alveolar walls are still defined, the interior of the alveoli being filled with a mass of cells, chiefly leucocytes. Bacilli are very abundant in these situations; they also occur, though in relatively scanty numbers, in the walls of patent alveoli. Amongst all the larger groups of bacilli leucocytes are very numerous and often contain bacilli.

Liver.

The tissue is largely replaced by ramifying caseous areas amongst which bacilli are numerous, though less abundant than in the lungs.

Kidneys.

There are small patches of interstitial infiltration, in which bacilli occur in scanty numbers.

Spleen.

Most of the tissue is replaced by caseous material. Bacilli are abundant.

Bronchial Gland.

The gland examined is caseous almost throughout, and is swarming with bacilli.

SUMMARY.

Acute, disseminated infection in all the organs except the kidneys; abundant multiplication of bacilli.

(3.)

VIRUS—P LIV.

Animal Inoculated.—G.P. 2572.

G.P. 2572 was inoculated intraperitoneally with ·1 mg. of culture of P LIV, and died 21 days afterwards.

Lungs.

Tubercles are not numerous. Those present are small, and either have not commenced to caseate or are only slightly caseous. Bacilli are scanty.

Liver.

The greater portion of the tissue is replaced by ramifying caseous areas. Bacilli are present in all parts of the tissue, but nowhere form large groups.

Kidneys.

Slight infection, identical with that found in G.P. 2571.

Spleen.

There are large caseous areas and many patches of early caseation. Leucocytes are very abundant in the lesions. In the older lesions bacilli are fairly abundant; they are rather scanty elsewhere.

SUMMARY.

The liver, and to a rather less degree the spleen, are very severely affected; the lungs and kidneys only slightly. Bacilli are much less numerous than in G.P. 2571.

(4.)

VIRUS—P LV.

Animal Inoculated.—G.P. 2573.

G.P. 2573 was inoculated subcutaneously with ·1 mg. of culture of P LV, and died 36 days afterwards.

Lungs.

Small tubercles are distributed throughout the tissue and appear to be extending along the alveolar walls. The lesions are not definitely caseous, but many of them are crowded with leucocytes. Bacilli are numerous in all the lesions.

Liver.

The liver is riddled with necrotic areas. Some of these are completely caseous; others are filled with masses of breaking down leucocytes. Bacilli are very abundant.

Kidneys.

No lesions or bacilli have been found in the specimen examined.

Spleen.

The spleen is in the same condition as the liver.

SUMMARY.

The lungs, liver, and spleen are acutely infected, but the lungs less extensively than the liver and spleen. No lesions or bacilli have been found in the kidneys.

(5.)

VIRUS—P LV.

Animal Inoculated.—G.P. 2574.

G.P. 2574 was inoculated intraperitoneally with ·1 mg. of culture of P LV, and died 19 days afterwards.

Lungs.

The lungs are in the same condition as those of G.P. 2573.

Liver.

The liver shows diffuse caseo-necrotic change and is almost as extensively damaged as the liver of G.P. 2573, but the bacilli have multiplied less freely.

Kidneys.

No lesions or bacilli have been found in the specimen examined.

Bronchial Gland.

The gland contains large areas of early caseation, in which bacilli are numerous.

SUMMARY.

Acute infection with particularly severe incidence on the liver.

General Results.

The guinea-pigs examined were, with one exception, inoculated with viruses possessing high virulence for the rabbit or calf. But as regards histological features and the distribution of bacilli, the lesions produced do not differ from those found in guinea-pigs inoculated with human bacilli possessing only low virulence for the rabbit and calf. With both classes of material severe disease is usually produced in most of the organs; but the kidneys, as a rule, either show relatively little disease or may escape entirely; they do not seem to be more susceptible to the porcine than to the human viruses.

But while there is this identity of histological features at the period when a fatal infection has been produced, the difference of virulence in the infective material is seen by comparing the periods of survival. These are much longer after inoculations with the less virulent material.

V.—LESIONS IN OTHER ANIMALS.

(1.)

VIRUS—P XLI.

Animal Inoculated.—Fowl 128.

Fowl 128 was inoculated intravenously with 15 mg. of culture of P XLI, and was killed 103 days afterwards.

Lungs and Kidneys.

No evidence of infection has been found.

Liver and Spleen.

There are a few minute suspicious foci, but no bacilli have been found in them.

SUMMARY.

The animal seems to have completely resisted infection.

(2.)

VIRUS—P XLI.

Animal Inoculated.—Fowl 186.

Fowl 186 was inoculated intraperitoneally with 100 mg. of culture of P XLI, and was killed 68 days afterwards.

Lungs.

There are a few small foci consisting mainly of fibroblasts and lymphocytes, which may have been produced by tubercle bacilli, but no bacilli are demonstrable.

Liver.

There are many small foci consisting of aggregations of lymphocytes, together with a smaller number of leucocytes, but no tubercle bacilli have been found in any of them.

Kidneys and Spleen.

No lesions or bacilli have been found.

Cervical Gland.

In the centre of one of the glands found in the neck there is a caseous mass which contains numerous tubercle bacilli identical in appearance with serum grown bacilli. There are also cell fusions bearing some resemblance to giant cells.

SUMMARY.

Neither tubercle bacilli nor tubercles have been found in the lungs, liver, kidneys, or spleen.

(3.)

VIRUS—P V.

Animal Inoculated.—Rat 76.

Rat 76 was inoculated intraperitoneally with 25 mg. of culture of P V, and died 23 days afterwards.

Lungs.

Groups of bacilli are present in all parts of the lung, but have not led to the formation of tubercles. Bacilli are present in the blood-vessels. Both in the blood-vessels and amongst the tissue cells bacilli are found within or surrounded by multinuclear leucocytes.

Liver.

Bacilli have grown into small colonies in all parts of the liver, but have caused very little tissue destruction. Giant cells are present. The bacilli are frequently intracellular.

Kidneys.

Bacilli are distributed all over the tissue, and in some places have grown into colonies. They have caused no tissue destruction.

Spleen.

Bacilli are abundant in all parts of the tissue. With the exception of a few giant cells, no lesions suggestive of tuberculosis are present.

SUMMARY.

Bacilli are distributed all over the body and have multiplied abundantly, but have not produced lesions suggestive of tuberculosis.

D.—LESIONS PRODUCED BY EQUINE TUBERCLE BACILLI.

I.—LESIONS IN HORSES.

VIRUS—E I.

Animal Inoculated.—Pony 16.

Pony 16 was inoculated intravenously with 10 mg. of culture of E I, and died 17 days afterwards.

Lungs.

The alveolar walls are thickened, and there are many small patches of consolidation. Some of these patches contain giant cells, and there is frequently a central focus where the tissue is commencing to break down. Leucocytes are abundant in the lesions. Bacilli occur in all parts of the tissue, and are plentiful in the lesions.

Liver.

There are no definite tubercles, but there are many minute foci showing slight cellular disintegration. Bacilli are fairly plentiful in these situations; they also occur occasionally in situations histologically normal.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Bacilli are distributed throughout the tissue in small numbers, and have produced minute foci of caseation.

Popliteal Gland.

A few bacilli are present within a giant cell. No other lesions have been found.

SUMMARY.

General dissemination; not enough tissue change to account for death.

VIRUS—E II.

Animal Inoculated.—Pony 18.

Pony 18 was inoculated intravenously with 10 mg. of culture of E II, and died 40 days afterwards.

Lungs.

There are large patches of consolidation in which the alveoli are completely filled up with leucocytes. The lesions bear no resemblance to the tubercular type of pneumonia. Tubercle bacilli are present in scanty numbers.

Liver.

Tubercle bacilli are distributed throughout the tissue in fairly large numbers. They have caused slight disintegration of the liver cells, together with an infiltration of leucocytes and lymphocytes, but have not led to tubercle formation. Long, thick bacilli which are not acid-fast are also present in part of the specimen but are not in association with the tubercle bacilli.

Kidneys.

There are several small deposits of bacilli, both within glomeruli and in the interstitial tissue. They have caused very little tissue damage.

Spleen.

Bacilli are scattered throughout the tissue in small numbers and in a few places have caused slight caseous change.

Bronchial Gland.

Bacilli are very abundant, but have not caused the formation of tubercles. Where they form very large groups there are masses of leucocytes and the remains of these cells. But in many parts of the section the bacilli have caused very little tissue change. There are a few giant cells.

SUMMARY.

The bacilli are generally disseminated, but have produced only slight tissue change.

VIRUS—E II.

Animal Inoculated.—Pony 20.

Pony 20 was inoculated subcutaneously with 50 mg. of culture of E II, and was killed 130 days afterwards.

Lungs.

In the lungs there are a few small foci which are overrun with lymphocytes and a few leucocytes but have not the characters of definite tubercles. No bacilli have been found.

Other Organs.

In the specimens of liver kidney, spleen, and a popliteal gland neither tubercles nor bacilli have been found.

SUMMARY.

With the exception of some suspicious foci in the lungs there is no evidence of dissemination. No tubercle bacilli have been found.

VIRUS—E IV.

Animal Inoculated.—Pony 22.

Pony 22 was inoculated subcutaneously with 50 mg. of culture of E IV, and was killed 125 days afterwards.

Lungs.

In the lungs there are irregular patches of consolidation which are infiltrated with numerous lymphocytes, contain giant cells, and show small patches of commencing caseation. Bacilli are moderately plentiful.

Liver.

There are many small tubercles, infiltrated with leucocytes and lymphocytes in about equal proportions and containing bacilli in scanty numbers.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are small, slightly caseous lesions in which giant cells occur. Bacilli are rare.

Mesenteric Gland.

Bacilli are disseminated throughout the gland in very large numbers. They have not produced definite areas of caseation, but have provoked a copious infiltration of leucocytes, many of which contain bacilli.

Popliteal Gland.

Bacilli are disseminated in moderate numbers throughout the gland, and have produced slightly caseous foci together with numerous giant cells. The infiltration of leucocytes is relatively small, compared with the mesenteric gland.

Heart Muscle.

There are fairly large patches of infiltration with leucocytes and lymphocytes which contain a few bacilli but the lesions can hardly be described as definite tubercles.

Local Lesion.

Bacilli have grown into small colonies in most parts of the specimen. In this respect the specimen is in contrast with local lesions of the chronic type. On the other hand, there is an absence of the progressive caseation and tissue destruction which characterise local lesions of an acute type. The lesion presents an abundant infiltration of leucocytes, lymphocytes, and plasma cells.

SUMMARY.

General dissemination, characteristic of infection intermediate in severity between the acute and the chronic.

VIRUS—E IV.

Animal Inoculated.—Pony 24.

Pony 24 was inoculated intravenously with 10 mg. of culture of E IV, and died 98 days afterwards.

Lungs.

The tissue shows thickening and infiltration of the alveolar walls, together with small patches of consolidation. The consolidated areas contain giant cells, lymphocytes, and leucocytes, and show a tendency to fibroid change; within these areas tubercle bacilli are present in small numbers.

Liver, Kidneys, and Spleen.

There are small lesions of doubtful nature, but no tubercle bacilli have been found. Other bacteria, both bacilli and cocci, are numerous throughout the specimens.

Popliteal Gland.

A few giant cells are present, but no tubercle bacilli have been found.

SUMMARY

A few bacilli are present in the lungs, and have produced small, apparently retrogressive lesions. No bacilli have been found elsewhere.

General Results.

After intravenous inoculation in doses of 10 mg., the bacilli have produced only slight tissue changes, and have not led to the formation of tubercles. In most of the organs examined bacilli are present, but they do not, as a rule, show signs of multiplication; in the bronchial gland of Pony 18, however, bacilli are present in enormous numbers.

In Pony 20 a dose of 10 mg. inoculated subcutaneously, has produced slight, apparently retrogressive infection of the lungs.

In Pony 22, however, the subcutaneous inoculation of 50 mg. produced general dissemination, with lesions intermediate between the acute and chronic types.

II.—LESIONS IN CALVES.

VIRUS—E I.

(1.)

Animal Inoculated.—Calf 436.

Calf 436 was inoculated subcutaneously with 50 mg. of culture of E I, and died 16 days afterwards. The cause of death was not determined.

Lungs.

The tissue contains many small foci which are partially necrotic and have no definite boundary zone.

These lesions contain numerous oxyphil leucocytes and a sprinkling of plasma cells is found at their periphery. No giant cells are present and there is no definite increase of the connective tissue elements. Bacilli are fairly numerous in the lesions.

Liver.

There are many small, partially necrotic foci similar to those present in the lungs. Bacilli are rather less numerous than in the lungs.

Kidneys.

No lesions or bacilli have been found.

Mediastinal Gland.

There are several small, partially necrotic foci containing numerous bacilli.

SUMMARY.

An early stage of acute disseminated tuberculosis.

(2.)

Animal Inoculated.—Calf 522.

Calf 522 was inoculated subcutaneously with 50 mg. of culture of E I, and died 36 days afterwards.

Lungs.

There is a patchy, caseous pneumonia. The lesions are evidently progressive and contain numerous bacilli. In some of the lesions the bacilli are no larger than serum grown bacilli. There is a deposit of fibrin.

Liver.

Miliary tubercles are numerous. They are extensively caseous and exhibit no signs of a demarcation boundary. Bacilli are moderately numerous.

Kidneys.

There are several small, caseous tubercles containing numerous bacilli.

Popliteal Gland.

There are many caseous tubercles which are crowded with bacilli.

Suprarenal Capsule.

Small tubercles are present and are commencing to caseate. Bacilli are less numerous than in the other tissues.

SUMMARY.

Acute disseminated tuberculosis.

(3.)

Animal Inoculated.—Calf 528.

Calf 528 was inoculated subcutaneously with 50 mg. of culture of E I, and died 30 days afterwards.

Lungs.

The tissue is packed with miliary tubercles which are becoming confluent and are crowded with bacilli.

Liver.

Caseous miliary tubercles are distributed in large numbers throughout the tissue. Bacilli are much less numerous than in the lungs.

Kidneys.

A few caseous tubercles are present and are surrounded with a broad peripheral zone amongst which plasma cells are numerous. Bacilli are not very plentiful.

Spleen.

The spleen is riddled with ramifying caseous foci. Bacilli are more numerous than in the liver or kidneys but much less abundant than in the lungs.

Lymphatic Glands.

A mediastinal gland is extensively caseous and contains numerous bacilli. In a popliteal gland the lesions are of the same type, but fewer in number.

Suprarenal Capsule.

Minute caseous foci are present. . Bacilli are rare.

SUMMARY.

Acute disseminated tuberculosis.

VIRUS—E II.

(1.)

Animal Inoculated.—Calf 406.

Calf 406 was inoculated subcutaneously with 10 mg. of culture of E II (from original material), and was killed 91 days afterwards.

Lungs.

Two specimens have been examined. Both contain a few caseous tubercles, the outer areas of which contain numerous lymphocytes and some plasma cells and leucocytes, but show no definite evidence of fibroid change. Giant cells are occasionally present. There are also several patches which show marked thickening and cellular infiltration of the alveolar walls. In the caseating tubercles bacilli are very numerous; they are also present in moderately large numbers in some of the infiltrated areas which have not commenced to caseate.

Liver.

In the specimen examined no tubercles or bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

Mesenteric Gland.

The gland contains caseous tubercles which have a well marked peripheral zone of fibrous tissue and contain many giant cells. Irregular areas, both large and small, are also present which have no boundary zone, are at the commencement of caseation, and contain many giant cells. Tubercle bacilli are very numerous and have a slight tendency to form circular groups with a clear space in the centre.

SUMMARY.

Lesions are present in the lungs and contain numerous bacilli; they are apparently progressive or at least cannot be regarded as retrogressive. Bacilli are also very numerous in the mesenteric gland.*

(2.)

Animal Inoculated.—Calf 506.

Calf 506 was inoculated subcutaneously with 50 mg. of culture of E II (from original material), and was killed 99 days afterwards.

Lungs, Liver, and Kidneys.

In the specimens examined neither tubercle bacilli nor lesions definitely suggestive of tuberculosis have been found.

Portal Gland.

There are many small, slightly caseous foci, most of which contain giant cells. Tubercle bacilli are present in several of the lesions but are scanty.

SUMMARY.

Only slight evidence of dissemination.

(3.)

Animal Inoculated.—Calf 578.

Calf 578 was inoculated subcutaneously with 50 mg. of culture of E II (from Calf 504), and was killed 90 days afterwards.

Lungs.

The lungs contain lesions which to a large extent are filled up with lymphocytes, but show small central areas of caseation; they are evidently retrogressive. Bacilli are present but rare.

Other Organs.

Neither lesions nor bacilli have been found in the specimens of liver, kidney, spleen, and a popliteal gland.

SUMMARY.

Mild, retrogressive infection of the lungs.

(4.)

Animal Inoculated.—Calf 580.

Calf 580 was inoculated subcutaneously with 50 mg. of culture of E II (from Calf 504), and died 29 days afterwards.

Lungs.

The lungs are semi-solid and contain ramifying caseous areas which are obviously progressive, and are not marked off into definite tubercles. Bacilli are very abundant.

Liver.

The liver contains many caseous lesions which are typical of acute infection and contain numerous bacilli.

Kidneys.

Several breaking down tubercles are present. Bacilli are abundant.

Spleen.

The tissue is largely replaced by ramifying caseous tracts which contain very numerous bacilli.

Popliteal Gland.

The gland contains several caseous patches in which bacilli are fairly numerous.

SUMMARY.

Typical of highly acute infection.

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* In the companion animal, Calf 404, chronic tubercles containing few bacilli were found in the bronchial glands; but no tubercles or bacilli were observed in the specimens of lung, liver, and kidney.

(5.)

Animal Inoculated.—Calf 592.

Calf 592 died 6 days after subcutaneous inoculation with 50 mg. of culture of E II (from Calf 506).

Lungs.

The lungs are congested and show a leucocytic infiltration, but no tubercle bacilli have been found.

Liver and Kidneys.

No lesions or bacilli have been found.

Spleen.

There are no lesions, but a group of four tubercle bacilli has been found.

Prescapular Gland.

No lesions have been found, and only three bacilli.

SUMMARY.

Slight evidence of dissemination.

(6.)

Animal Inoculated.—Calf 594.

Calf 594 died 16 days after subcutaneous inoculation with 50 mg. of culture of E II (from Calf 506).

Lungs.

One portion of lung shows the non-tubercular type of pneumonic consolidation and contains very few tubercle bacilli. Another specimen shows numerous, typical, miliary tubercles. These contain large numbers of bacilli and are evidently confluent.

Liver.

Small caseous tubercles are present. It is doubtful whether they are progressive or quiescent in character. They have not a well-marked boundary zone, but on the other hand they only contain small numbers of bacilli.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are patches of slight caseation containing moderate numbers of bacilli.

Bronchial Gland.

A bronchial gland contains many caseous patches in which bacilli are abundant.

SUMMARY.

General dissemination. The lesions in the lungs are acute in type.

(7.)

Animal Inoculated.—Calf 596.

Calf 596 was inoculated subcutaneously with 50 mg. of culture of E II (from Calf 406), and was killed 90 days afterwards.

Lungs.

The lungs contain fairly large patches of consolidation which are semi-caseous and appear to be slowly progressive. These lesions contain tubercle bacilli in moderately large numbers.

Liver.

There are a few minute foci of infiltration with leucocytes and lymphocytes; an occasional bacillus has been found in these areas.

Kidneys.

No lesion or bacilli have been found.

Spleen.

Two or three bacilli have been found; no definite tissue changes have been observed.

Popliteal Gland.

A few bacilli are present, but have produced no histological change.

SUMMARY.

Slowly progressive tuberculosis of the lungs; a few bacilli, but very little evidence of disease, in other organs.

(8.)

Animal Inoculated.—Calf 626.

Calf 626 was inoculated subcutaneously with 50 mg. of culture of E II (from Calf 504), and died 54 days afterwards.

Lungs.

The tissue is permeated with large, irregularly defined caseous areas which are obviously progressive. Bacilli are abundant, and the general features of the tissue are characteristic of acute infection.

Liver.

There are a few caseous lesions, progressive in type and containing fairly large numbers of bacilli.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The tissue is permeated with caseous, progressive lesions containing fairly numerous bacilli.

Popliteal Gland.

There are a few patches of early caseation containing a few bacilli.

SUMMARY.

Progressive, disseminated tuberculosis with severe incidence on the lungs.

(9.)

Animal Inoculated.—Calf 628.

Calf 628 was inoculated subcutaneously with 50 mg. of culture of E II (from Calf 506), and was killed 90 days afterwards.

Lungs.

There are partially confluent and apparently progressive patches of consolidation. Some of these are slightly caseous, but they do not present the rapidly progressive tissue destruction found in acute infections. The leucocytic infiltration is very abundant. Bacilli are scattered in moderate numbers throughout the specimen, but have not grown into groups.

Liver and Kidneys.

No lesions or bacilli have been found.

Spleen.

The spleen contains caseous tubercles, more or less definitely circumscribed, and approximating to the chronic type. Bacilli occur in the lesions in rather small numbers.

Popliteal Gland.

There are large caseous patches containing moderate numbers of bacilli. No giant cells are present. The infiltration of leucocytes is particularly abundant.

SUMMARY.

Moderately severe infection of lungs, spleen, and a popliteal gland.

(10.)

Animal Inoculated.—Calf 638.

Calf 638 was inoculated subcutaneously with 50 mg. of culture of E II (from Calf 596), and was killed 40 days afterwards.

Lungs.

The specimen of lung is semi-solid, and contains large ramifying patches of caseation. The lesions are acute in type and contain fairly numerous bacilli.

Liver.

The liver contains several small, caseous tubercles of a chronic type, and also isolated giant cells. Bacilli are rather scanty.

Kidneys.

There are many caseous tubercles and patches of infiltration in which bacilli are present in rather small numbers.

Spleen.

There are extensive, ramifying tracts of caseation. The lesions are obviously progressive, but bacilli are not very numerous.

Popliteal Gland.

The gland is to a large extent replaced by caseous material. Bacilli are fairly plentiful.

SUMMARY.

General dissemination; the lesions in the lungs are typical of acute infection.

VIRUS—E III.

(1.)

Animal Inoculated.—Calf 440.

Calf 440 was inoculated subcutaneously with 50 mg. of culture of E III, and died 72 days afterwards.

Lungs.

The tissue is in a semi-solid condition and is occupied by irregular and extensive necrotic tracts. There is no limitation into tubercles and no evidence of conservative change. No giant cells are present. There are masses of multinuclear leucocytes and their remains at the margin of the necrotic areas, and external to these cells there is a conspicuous layer of plasma cells. There is an abundant deposit of fibrin. Tubercle bacilli are distributed throughout the tissue in large numbers and have grown into large groups both in the caseous areas and in other parts of the tissue where caseation has not commenced. Groups of multinuclear leucocytes are generally present in close association with the bacilli. The bacilli are from about 1·5 to 3 μ in length, and occasionally rather longer. About half the bacilli are somewhat curved. The staining is uniform.

Liver.

Two specimens have been examined. In one of them the only lesion present is a small, definitely circumscribed tubercle in an advanced stage of caseation. This tubercle contains a moderately large number of bacilli. In the second specimen there are numerous lesions. These lesions are partially necrotic and often contain giant cells; but they have often little or no peripheral zone, and their outlines are very irregular; it is evident that they are more rapidly progressive than the lesion found in the first specimen. All the lesions contain bacilli in moderate numbers, and in some of them there is a deposit of fibrin.

Kidneys.

In one specimen no lesions or bacilli are present. In a second specimen there are several tubercles. Some of these are large and extensively caseous. Surrounding the caseous material is a mass of oxyphil leucocytes and their remains, and external to this layer there is a well-marked infiltration of plasma cells and small lymphocytes. The smaller lesions are of the same type, but are only at the commencement of caseation. Tubercle bacilli are very abundant, particularly in the larger tubercles.

Right Prepectoral Gland.

The gland is extensively caseous and contains dense masses of bacilli.

SUMMARY.

General tuberculosis with highly acute infection of the lungs. Many tubercles, containing very large numbers of bacilli, are present in the kidneys.

(2.)

Animal Inoculated.—Calf 508.

Calf 508 was inoculated subcutaneously with 50 mg. of culture of E III, and was killed, when moribund, 73 days afterwards.

Lungs.

The tissue is nearly completely consolidated, is extensively caseous, and contains large numbers of bacilli. Its condition is identical in every respect with that produced by the subcutaneous inoculation of 50 mg. of a virulent human or bovine virus. There is a deposit of fibrin.

Liver.

There are many minute, caseating tubercles, some of which contain giant cells. Bacilli are very scanty.

Kidneys.

Large caseating tubercles are present and show a well marked periphery of plasma cells. Bacilli are numerous.

Spleen.

There are many small caseous foci, in which bacilli are fairly numerous.

Popliteal Gland.

Caseous tubercles are present. They have a well marked fibrous zone and contain numerous giant cells. Bacilli are fairly numerous.

SUMMARY.

The lungs and kidneys are extensively affected and contain numerous bacilli. The other organs are affected less severely. On the whole, the infection is rather less severe than that usually produced by 50 mg. of a virulent human or bovine virus.

VIRUS—E IV.

(1.)

Animal Inoculated.—Calf 410.

Calf 410 was inoculated subcutaneously with 10 mg. of culture of E IV (from original material), and was killed 88 days afterwards.

Lungs.

No tubercles or tubercle bacilli have been found. There is a definite leucocytosis, but no consolidation of the tissue.

Liver and Kidneys.

No tubercles or bacilli have been found.

Bronchial Gland.

There is a small fibro-caseous tubercle containing a few bacilli.

SUMMARY.

With the exception of a small tubercle, chronic in type, found in a bronchial gland, there is no evidence of dissemination in the specimens examined.

(2.)

Animal Inoculated.—Calf 498.

Calf 498 was inoculated subcutaneously with 50 mg. of culture of E IV (from original material), and died 89 days afterwards.

Lungs.

The lungs are acutely infected. They contain many extensive ramifying tracts of caseation, and the tissue external to the lesions is highly congested. Bacilli are abundant in all parts of the tissue and form numerous colonies visible with the low power. The bacilli often form a solid ring, surrounding a cell nucleus. The colonies of bacilli often form groups ; it seems as though large numbers of bacilli must have been carried to the particular places from which these groups originated. A similar appearance is sometimes seen after intravenous inoculation. No fibrin is demonstrable.

Liver and Spleen.

No lesions or bacilli have been found.

Kidneys.

There is some interstitial nephritis of a non-tubercular character. No bacilli are present.

Popliteal Gland.

A few early caseous foci containing a few bacilli are present.

SUMMARY.

Acute infection of the lungs, with very large numbers of bacilli, suggestive of intravenous inoculation. Very little dissemination elsewhere.

(3.)

Animal Inoculated.—Calf 502.

Calf 502 was inoculated subcutaneously with 50 mg. of culture of E IV (from original material), and was killed 90 days afterwards.

Two pieces of lung and pieces of liver, kidney, spleen, and a popliteal gland have been examined. In none of these have tubercle bacilli been found or lesions definitely suggestive of tuberculosis.

SUMMARY.

No evidence of tuberculosis in the specimens examined.

(4.)

Animal Inoculated.—Calf 582.

Calf 582 was inoculated subcutaneously with 50 mg. of culture of E IV (from Calf 408), and was killed 88 days afterwards.

Lungs.

The tissue contains a few small tubercles which are discrete, abundantly infiltrated with lymphocytes, and appear to be retrogressive. A few bacilli are present in the lesions.

Liver.

The specimen shows a few small, caseous tubercles which are surrounded by fibrous elements and contain giant cells. Bacilli are rare.

Kidneys.

No lesions or bacilli have been found in the specimen examined.

Spleen.

The spleen contains caseous lesions in which bacilli are plentiful. The lesions show no definite boundary zone but appear to be expanding. Bacilli are found at their periphery.

Mediastinal Gland.

There are a few minute caseous foci, but no bacilli have been found.

SUMMARY.

Progressive tubercles are present in the spleen ; in the other organs examined the lesions are few in number and appear to be quiescent or retrogressive.

(5.)

Animal Inoculated.—Calf 586.

Calf 586 was inoculated subcutaneously with 50 mg. of culture of E IV (from Calf 498), and died 36 days afterwards.

Lungs.

There are a few suspicious foci showing giant cells, but neither definite tubercles nor tubercle bacilli have been found. There are several patches of partial consolidation which are crowded with leucocytes and are not suggestive of tuberculosis.

Other Organs.

In the specimens of lung, liver, kidney, spleen, and a mediastinal gland neither tubercles nor tubercle bacilli have been found.

SUMMARY.

No definite evidence of dissemination.

(6.)

Animal Inoculated.—Calf 602.

Calf 602 was inoculated subcutaneously with 50 mg. of culture of E IV (from Calf 502), and was killed 90 days afterwards.

Lungs.

The specimen of lung is semi-solid. The centres of the consolidated areas are caseous, but the peripheral regions show a tendency to conservative change, containing many fibroblasts in addition to leucocytes and lymphocytes. Bacilli are scanty.

Liver.

No lesions or bacilli have been found in the specimen examined.

Kidneys.

There are large, caseous, and apparently slowly progressive tubercles in which bacilli are scanty.

Spleen.

The spleen contains discrete, caseous tubercles, with a slight tendency to calcification in their centres. The lesions contain only few bacilli, are definitely of a chronic type, and probably retrogressive.

Precrural Gland.

No lesions or bacilli have been found.

SUMMARY.

Disseminated tuberculosis of a chronic type, but probably slowly progressive in the lungs and kidneys.

(7.)

Animal Inoculated.—Calf 614.

Calf 614 was inoculated subcutaneously with 50 mg. of culture of E IV (from Calf 502), and died 33 days afterwards.

Lungs.

The lungs are semi-solid, being occupied with extensive, ramifying, caseous areas in which tubercle bacilli are abundant. The infection is severe and evidently progressive.

Liver and Kidneys.

No lesions or bacilli have been found.

Mediastinal Gland.

The gland contains many small, caseous, and apparently progressive foci, in which bacilli are fairly numerous.

SUMMARY.

Severe progressive infection of the lungs.

(8.)

Animal Inoculated.—Calf 620.

Calf 620 was inoculated subcutaneously with 50 mg. of culture of E IV (from original material), and was killed 91 days afterwards.

Lungs, Liver, and Kidneys.

Neither tubercles nor tubercle bacilli have been found in the specimens examined.

Spleen.

There are a few foci which are slightly caseous and contain giant cells. In one of these lesions a tubercle bacillus has been found.

Popliteal Gland.

No lesions or bacilli have been found.

SUMMARY.

With the exception of the spleen, where there is very slight disease, the specimens show no evidence of dissemination.

(9.)

Animal Inoculated.—Calf 630.

Calf 630 was inoculated subcutaneously with 50 mg. of culture of E IV (from Calf 502), and died 31 days afterwards.

Lungs.

The specimen of lung is almost solid and contains many patches of breaking down tissue. Tubercle bacilli occur in all parts of the specimen and are numerous in the breaking down areas.

Liver.

Caseous, miliary tubercles, acute in type and containing large numbers of bacilli, are scattered abundantly throughout the tissue.

Kidneys.

There are several progressive, interstitial lesions containing moderately large numbers of bacilli.

Spleen.

There are extensive, ramifying caseous tracts, in which bacilli are very abundant.

Popliteal Gland.

The gland is extensively caseous and contains large numbers of bacilli.

SUMMARY.

Acute disseminated tuberculosis.

General Results.

The virus E I produces in calves acute disseminated tuberculosis which is identical in histological characters with that caused by highly virulent human, bovine, or porcine bacilli.

The results with E II are irregular, and may be summarised as follows :—

(a) *Cultures from Original Material.*— Calf 406 received a dose of 10 mg. ; the lesions in the lungs, though not acute in type, contain numerous bacilli and cannot be regarded as retrogressive. But in the companion animal, Calf 404, which received the same dose, only very slight evidence of disease was found. And in Calf 506, which received a dose of 50 mg., there was only slight evidence of dissemination.

(b) *Cultures from Calves inoculated with Original Material.*—(1) 50 mg. of culture from Calf 406 were inoculated into Calf 596 and produced slowly progressive tuberculosis of the lungs, but very little evidence of disease in the other organs examined. (2) Doses of 50 mg. of culture from Calf 504 (in which 50 mg. of culture from original material produced only slight disease) were inoculated into Calves 578, 580, and 626. In Calf 578 mild, retrogressive infection was produced. In Calf 580, which died in 29 days, the lesions were typical of highly acute infection. Calf 626 died in 54 days with progressive, disseminated tuberculosis, the lungs being typical of acute infection. (3) Doses of 50 mg. of culture from Calf 506 were inoculated into Calves 592, 594, and 628. In Calf 592 there was only slight evidence of dissemination. Calf 594 died in 16 days and showed general dissemination, the lesions in the lungs being acute in type. Calf 628, killed 90 days after inoculation, exhibited moderately severe infection of the lungs and spleen.

(c) *A Culture obtained after two passages in Calves.*— A dose of 50 mg. of culture from Calf 596 (inoculated from culture of Calf 406) was inoculated into Calf 638. The animal was killed 40 days afterwards and showed general dissemination, the lesions in the lungs being typical of acute infection.

The two calves inoculated with E III show typically acute infection of the lungs and general dissemination, but less severe disease of the liver than is usual in very acute cases. The animals survived the inoculation for 72 and 73 days respectively, periods which are rather longer than the average.

The results with E IV are irregular, and may be summarised as follows :—

(a) *Cultures from Original Material.*—In Calf 410, inoculated with 10 mg., there was only very slight evidence of dissemination. In Calf 502, inoculated with 50 mg., no evidence of tuberculosis was found in the specimens examined. In Calf 620, also inoculated with 50 mg., a few small lesions in the spleen were the only evidence of disease found. But in Calf 498, which died 89 days after the subcutaneous inoculation of 50 mg., there was acute infection of the lungs, with very large numbers of bacilli, suggestive of intravenous inoculation, but very little dissemination elsewhere.

(b) *Cultures from Calves inoculated with Original Material.*—(1) 50 mg. of culture from Calf 408 produced in Calf 582 disseminated tubercles which appeared to be progressive in the spleen but retrogressive in the other tissues examined. (2) 50 mg. of culture from Calf 498 failed to produce any evidence of dissemination in the tissues examined of Calf 586. (3) Doses of 50 mg. of culture from Calf 502 were inoculated into Calves 602, 614, and 630. In the first animal disseminated tuberculosis of a chronic, but probably slowly progressive type was produced. The second animal died in 33 days with acute tuberculosis of the lungs. The third exhibited acute disseminated tuberculosis.

III.—LESIONS IN RABBITS.

VIRUS—E I.

(1.)

Animal Inoculated.—Rabbit 934.

Rabbit 934 was inoculated subcutaneously with ·1 mg. of culture of E I, and died 75 days afterwards.

Lungs.

The tissue is almost completely consolidated and contains many irregular and obviously progressive areas of caseation. Apart from these areas the tissue consists of a confused mass of desquamated alveolar epithelium, leucocytes, lymphocytes, plasma cells, and fibroblasts. The last three types of cells are numerous and indicate a greater amount of tissue resistance than is found in very acute infections. Bacilli are plentiful everywhere and in many places have grown into groups.

Liver.

Minute tubercles are disseminated throughout the tissue in large numbers. They are only slightly caseous and are infiltrated with lymphocytes and leucocytes; in some of them giant cells are present. Bacilli are scanty.

Kidneys.

Caseating, progressive tubercles are present and contain numerous bacilli.

Inguinal Gland.

The gland shows diffuse caseation and contains numerous bacilli.

SUMMARY.

Progressive infection with particularly severe incidence on the lungs.

(2.)

Animal Inoculated.—Rabbit 1309.

Rabbit 1309 was inoculated intravenously with ·1 mg. of culture of E I, and died 19 days afterwards.

Lungs.

The lungs are in an advanced stage of caseous pneumonia, with small patches of air-containing tissue left between the consolidated areas. Bacilli are numerous everywhere, and in the centres of many of the caseous patches have grown into dense masses.

Liver.

The tissue is riddled with ramifying, caseous tubercles containing numerous bacilli. Many giant cells are present.

Kidneys.

Small tubercular lesions, interstitial in origin and containing many bacilli, are scattered throughout the cortex.

Spleen.

Bacilli are numerous in all parts of the tissue and have produced early caseation.

Mediastinal Gland.

The cortical portion of the gland is completely caseous and is swarming with bacilli.

SUMMARY.

Acute disseminated tuberculosis.

VIRUS—E II.

(1.)

Animal Inoculated.—Rabbit 1082.

Rabbit 1082 was inoculated subcutaneously with 1 mg. of culture of E II (from original material), and was killed 102 days afterwards.

Lungs.

There are several consolidated foci which are of considerable size and have a broad but irregular cellular periphery. Most of these areas are caseous in the centre. Tubercle bacilli are scanty.

Liver, Spleen, and Kidneys.

No lesions or bacilli have been found.

Spinal Cord.

In the specimen examined the greater part of the tissue is replaced by caseous material in which tubercle bacilli are abundant.

SUMMARY.

Caseous tubercles of a chronic type in the lungs; advanced caseation of the spinal cord. No lesions found in liver, kidneys, or spleen.

(2.)

Animal Inoculated.—Rabbit 1083.

Rabbit 1083 was inoculated subcutaneously with 10 mg. of culture of E II (from original material), and was killed 102 days afterwards.

Lungs.

There is considerable thickening of the alveolar walls and some large, partially caseous tubercles are also present. Bacilli are very scanty and have only been found in the caseating tubercles.

Liver.

There are a few small patches of infiltration with lymphocytes and leucocytes but no definitely recognisable tubercles. No bacilli have been found.

Kidneys.

There are a few slightly caseous foci, in which bacilli are scantily distributed.

Spleen.

No lesions or bacilli have been found.

SUMMARY.

Chronic tuberculosis of the lungs with few bacilli; slight lesions in the kidneys; no definite lesions in the liver.

(3.)

Animal Inoculated.—Rabbit 1331.

Rabbit 1331 was inoculated intravenously with 1 mg. of culture of E II (from original material), and died 127 days afterwards.

Lungs.

The specimen of lung shows large, irregularly distributed patches of consolidation which are extensively caseous. The lesions contain numerous bacilli and are obviously progressive.

Liver.

The specimen of liver contains a small caseous tubercle in which no bacilli have been found.

Kidneys.

There are several tubercles showing caseous centres and a broad cellular periphery. They contain bacilli, but only in small numbers.

SUMMARY.

Severe, progressive infection of the lungs, with large numbers of bacilli; slight, and apparently quiescent infection of liver and kidneys.

VIRUS—E III.

Animal Inoculated.—Rabbit 1232.

Rabbit 1232 was inoculated subcutaneously with 10 mg. of culture of E III, and died 69 days afterwards.

Lungs.

The greater portion of the tissue is partly or completely solidified, and there are many large patches which are completely caseous. Bacilli are distributed throughout the tissue, and in many places have grown into groups and colonies visible under a low power.

Liver.

There are a few small caseous foci, in which no bacilli have been found. The largest of these foci has a broad fibrous periphery.

Kidneys.

There are two large, caseating tubercles which are bounded by, and partially infiltrated with, numerous plasma cells. Bacilli are numerous, and form dense masses in the remains of some of the tubules. Giant cells are present; in the case of some, the nuclei form a heaped up mass in the centre of the cell.

Spleen.

The spleen contains many areas which are filled with nuclear débris and contain large numbers of cocci and short bacilli. No acid-fast bacilli have been found.

SUMMARY.

Advanced, progressive tuberculosis of the lungs. Caseous tubercles, containing numerous bacilli, are also present in the kidneys.

VIRUS—E IV.

(1.)

Animal Inoculated.—Rabbit 1080.

Rabbit 1080 was inoculated subcutaneously with 20 mg. of culture of E IV (from original material), and was killed 100 days afterwards.

Lungs.

The specimen is typical of caseous pneumonia. Tubercle bacilli are rather scanty.

Liver.

There are several small tubercles which appear to be retrogressive in character. They contain giant cells and are infiltrated with lymphocytes. Bacilli are scanty on the whole, but some of the giant cells contain several.

Kidneys.

Tubercles of a chronic type are present. They contain few bacilli.

Spleen.

Patches of slight caseation are present. The lesions contain giant cells and are not acute in type, though not definitely retrogressive. Tubercle bacilli are scanty; cocci and short blue-staining bacilli are also present.

Spinal Cord.

Caseous areas, containing tubercle bacilli, are present.

SUMMARY.

Slowly progressive tuberculosis, with advanced lesions in the lungs; bacilli scanty.

(2.)

Animal Inoculated.—Rabbit 1418.

Rabbit 1418 was inoculated subcutaneously with 1 mg. of culture of E IV (from Calf 502), and died 78 days afterwards.

Lungs.

The lungs show large, irregular, confluent patches of advanced caseation. Bacilli are very abundant.

Liver.

There are a few minute, slightly caseous foci in which bacilli are scanty.

Kidneys.

There are large, caseous tubercles in which bacilli are numerous. The tubercles have a fairly well defined boundary zone of lymphocytes and fibroblasts, but bacilli are fairly frequent in this region and the lesions appear to be slowly progressive.

Spleen.

Bacilli are distributed in small numbers and have produced small, slightly caseous foci.

SUMMARY.

Advanced caseation of lungs and kidneys, with numerous bacilli ; slight infection of liver and spleen.

(3.)

Animal Inoculated.—Rabbit 1699.

Rabbit 1699 was inoculated intravenously with 1 mg. of culture of E IV (from original material), and died 32 days afterwards.

Lungs.

The lungs are nearly solid and are typical of tubercular pneumonia. Bacilli are very numerous.

Liver.

There are several small, progressive tubercles containing fairly large numbers of bacilli.

Kidneys.

There are numerous, progressive lesions containing large numbers of bacilli.

Spleen.

There are many caseous lesions containing numerous bacilli.

Bronchial Gland.

The gland contains caseous patches in which bacilli are very numerous.

SUMMARY.

Disseminated, progressive tuberculosis ; bacilli numerous.

VIRUS—E V.

Animal Inoculated.—Rabbit 1445.

Rabbit 1445 was inoculated subcutaneously with 10 mg. of culture of E V, and died 34 days afterwards.

Lungs.

The tissue is crowded with confluent, extensively caseous tubercles containing numerous bacilli.

Liver.

The liver is permeated with ramifying tubercular lesions. These show more tendency to conservative change than those in the lungs, and contain bacilli in relatively small numbers.

Kidneys.

A few bacilli are present, but no definite lesions have been produced.

Spleen.

There are ramifying caseous patches, containing numerous bacilli.

SUMMARY.

Progressive tuberculosis.

General Results.

The lesions produced by the viruses E I, E III, and E V, were identical with those caused by highly virulent human, bovine, and porcine viruses. The effects of subcutaneous inoculation were much less severe than those produced by intravenous inoculation.

E II and E IV have produced disseminated disease, but it was of a more chronic character in some cases, *e.g.*, in Rabbits 1082, 1083, and 1080, inoculated subcutaneously with culture from original material. But in Rabbit 1418, 1 mg., subcutaneously, of E IV (through Calf 502) produced severe disease. Intravenous inoculations of cultures from original material of E II and E IV produced advanced and fatal disease, but the infection was less acute in type than that caused, intravenously, by E I.

IV.—LESIONS IN LEMURS, MONKEYS, AND BABOONS.

VIRUS—E II.

(1.)

Animal Inoculated.—Lemur 16.

Lemur 16 was inoculated subcutaneously with 1 mg. of culture of E II (from original material), and died 35 days afterwards.

Lungs.

There is considerable thickening and infiltration of the alveolar walls, and throughout the tissue there are irregular patches of commencing consolidation which have no definite boundary zone and have not advanced to caseation. Oxyphil leucocytes and plasma cells are distributed irregularly and are not particularly numerous. No fibrin is demonstrable. In some of the consolidated areas bacilli are very numerous; in others they are relatively scanty. Amongst the walls of patent alveoli isolated bacilli are often to be found.

Liver.

There is a general infiltration with tubercle bacilli. In places where the bacilli are numerous the tissue is slightly necrotic and is invaded with leucocytes and lymphocytes, though not in large numbers; fibrin is present in these situations. Bacilli occur elsewhere in smaller numbers and are sometimes present within cells of the liver parenchyma.

Kidneys.

There is a general infiltration of the tissue with leucocytes, lymphocytes, and plasma cells, and in some situations the renal tubes are partially obliterated and necrosis is commencing. The plasma cells are particularly numerous throughout the interstitial tissue. No fibrin is present. Bacilli are abundant and are disseminated everywhere.

Spleen.

Bacilli are distributed abundantly in all parts of the tissue, and have produced areas of partial necrosis. Fibrin is present.

SUMMARY.

Acute disseminated tuberculosis.

(2.)

Animal Fed.—Lemur 18.

Lemur 18 was fed with 1 mg. of culture of E II (from original material), and died 76 days afterwards.

Lungs.

There are numerous small consolidated patches, some of which are commencing to caseate, and in many situations there is an irregular thickening of the alveolar walls. No giant cells have been noted. Oxyphil leucocytes are moderately abundant; some of them are coarsely granular. Plasma cells are distributed in all parts of the infiltrated and consolidated areas. No fibrin is demonstrable. Tubercle bacilli are present in nearly all parts of the tissue, and are abundant in some of the breaking down areas.

Liver.

The liver is permeated with numerous small areas which are commencing to caseate, have no definite demarcation zone, and occasionally contain a deposit of fibrin. Tubercle bacilli are present but rather scanty.

Kidneys.

There is a general interstitial infiltration which is characterised by the presence of a large number of plasma cells. In some situations the renal tubules are partially broken down; in these places groups of coarsely granular oxyphil leucocytes generally occur. Tubercle bacilli are present but rare.

Spleen.

The spleen contains many caseous areas which are not circumscribed. In a few of these areas bacilli are numerous, but elsewhere they are scanty.

SUMMARY.

Progressive disseminated tuberculosis.

(3.)

Animal Inoculated.—Monkey 168.

Monkey 168 was inoculated subcutaneously with 10 mg. of culture of E II (from original material), and was killed, when moribund, 51 days afterwards.

Lungs.

The specimen of lung contains several tubercles which are small and commencing to caseate, but show no signs of conservative change and contain fairly large numbers of bacilli. There is some generalised thickening of the alveolar walls, but the area occupied by definitely tubercular lesions is relatively small.

Liver.

Small tubercles, in an early stage of caseation and showing no signs of conservative change, are abundantly distributed. Bacilli are fairly numerous in the lesions.

Kidneys.

No lesions or bacilli have been found in the specimen examined.

Spleen.

There are many caseous areas which contain bacilli in fairly large numbers.

SUMMARY.

General dissemination in lungs, liver, and spleen. The lesions are not very advanced, but show no sign of conservative change and contain fairly large numbers of bacilli.

(4.)

Animal Inoculated.—Monkey 180.

Monkey 180 was inoculated subcutaneously with 1 mg. of culture of E II (from original material), and died 101 days afterwards.

Lungs.

The tissue is congested, but there are no lesions suggestive of tuberculosis, and no bacilli have been found.

Liver.

No lesions or bacilli have been found.

Kidneys.

The specimen contains a typical tubercle, but no bacilli have been found in it.

Spleen.

Typical tubercles of a chronic type are present. No bacilli have been found in them.

Axillary Gland.

A few small, suspicious foci are present. No bacilli have been found.

SUMMARY.

No tubercle bacilli have been found. Tubercles are present in the kidneys and spleen.

(5.)

Animal Inoculated.—Monkey 184.

Monkey 184 was inoculated subcutaneously with 0·1 mg. of culture of E II (from original material), and was killed 98 days afterwards.

Lungs.

The lungs are somewhat congested and contain small, slightly caseous foci in which bacilli are rare.

Liver.

The liver contains many small lesions which are retrogressive in character and are abundantly infiltrated with lymphocytes. Bacilli are rare.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are many fairly extensive lesions showing advanced caseation and containing moderately large numbers of bacilli.

Axillary Gland.

Small, caseous patches are present and contain a few bacilli.

SUMMARY.

Disseminated lesions of a mild type.

(6.)

Animal Fed.—Monkey 186.

Monkey 186 was fed with 1 mg. of culture of E II (from original material), and died 139 days afterwards.

Lungs.

No bacilli or lesions suggestive of tuberculosis have been found.

Liver.

There are several small lesions which are infiltrated with lymphocytes and leucocytes. In these, bacilli are occasionally present but are rare.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are several small, definitely caseous patches, in which no bacilli have been found.

Submaxillary Lymphatic Gland.

The gland is almost completely caseous and contains large numbers of bacilli.

SUMMARY.

There is only slight evidence of dissemination.

(7.)

Animal Fed.—Monkey 202.

Monkey 202 was fed with 60 mg. of culture of E II (from original material), and was killed 101 days afterwards.

Bronchial Gland.

The gland examined is extensively caseous. Tubercle bacilli are present, but scanty.

Other Organs.

Neither tubercles nor tubercle bacilli have been found in the specimens of lung, liver, kidney, and spleen.

SUMMARY.

Except in a bronchial gland, no evidence of tuberculosis has been found.

(8.)

Animal Fed.—Monkey 208.

Monkey 208 was fed with 50 mg. of culture of E II (from original material), and died 115 days afterwards.

Lungs.

There are several discrete, caseous tubercles with a fairly definite periphery of lymphocytes and fibroblasts. Bacilli are moderately numerous in the lesions.

Liver.

There are many minute tubercles which are apparently not of a progressive nature. Bacilli are rare.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are several small patches of early caseation. Bacilli are scanty.

Lumbar Gland.

There are caseous tubercles with a well-marked fibrous periphery. These lesions contain numerous bacilli, and bacilli are frequently found in the fibrous periphery.

SUMMARY.

Disseminated tuberculosis of a chronic type. In some of the chronic tubercles bacilli are multiplying freely.

(9.)

Animal Fed.—Monkey 212.

Monkey 212 was fed with 50 mg. of culture of E II (from Calf 404), and died 82 days afterwards.

Lungs.

There is a slight, patchy thickening of the alveolar walls, but no definite tubercles have been observed. Leucocytes are very numerous in the thickened areas. Bacilli are scattered about the specimen in small numbers.

Liver.

There are several suspicious foci of infiltration, and one or two bacilli have been found in some of them; but the lesions have not the definite structure of tubercles.

Kidneys and Spleen.

No lesions or bacilli have been found.

SUMMARY.

Slight, apparently non-progressive infection of the lungs and liver.

(10.)

Animal Inoculated.—Monkey 216.

Monkey 216 was inoculated subcutaneously with 1 mg. of culture of E II (from original material), and died 66 days afterwards.

Lungs.

There are several small, isolated patches of consolidation. A few of these are slightly caseous but the majority, whilst containing numerous lymphocytes and leucocytes and an increase of fibrous elements, show no sign of caseation. Bacilli are numerous, and in the larger lesions have grown into dense groups and colonies. Bacilli are also found throughout the tissue in places where there is no histological change.

Liver.

Bacilli are disseminated freely throughout the tissue and in many places are associated with small, slightly caseous foci.

Kidneys.

No definite tubercles have been found, but bacilli occur in rather small numbers in all parts of the tissue and some have been found within glomeruli.

Spleen.

The tissue is swarming with colonies of bacilli which have produced practically no tissue destruction.

Axillary Gland.

The gland is swarming with bacilli and contains large areas of early caseation.

SUMMARY.

Dissemination and abundant multiplication of bacilli with little tissue destruction; not unlike an intravenous inoculation with avian bacilli.

(11.)

Animal Inoculated.—Monkey 220.

Monkey 220 was inoculated subcutaneously with 1 mg. of culture of E. II (from Calf 408), and was killed 96 days afterwards.

Lungs.

The tissue contains many caseous, semi-confluent areas which appear to be slowly progressive. Bacilli are scanty.

Liver.

The liver contains many small discrete tubercles with retrogressive characters. Bacilli are scanty.

Kidneys.

There are a few small, retrogressive tubercles Only one bacillus has been found.

Spleen.

The spleen contains large caseous tubercles of a chronic type. Bacilli are rare.

Bronchial Gland.

There are a few foci of early caseation in which bacilli are scanty.

SUMMARY.

Disseminated tuberculosis of a mild type. Bacilli scanty.

VIRUS—E III.

Animal Inoculated.—Lemur 14.

Lemur 14 was inoculated subcutaneously with 1 mg. of culture of E III, and died 28 days afterwards.

Lungs.

Interspersed amongst normal or only slightly infiltrated alveoli there are many consolidated patches which are filled with cellular débris and are commencing to break down. These patches have no demarcation zone and contain no giant cells; occasionally a little deposit of fibrin is found within them. They are swarming with tubercle bacilli. Bacilli are also present in scanty numbers in the rest of the tissue.

Liver.

The liver is riddled with small tubercles which show no boundary zone but contain a fibrinous deposit and are commencing to break down. Bacilli are abundant.

Kidneys.

There are many patches of interstitial infiltration, some of which are commencing to break down. They contain bacilli in moderately large numbers. Plasma cells are conspicuous both in these areas and in the rest of the tissue. No fibrin is present.

Spleen.

The spleen is swarming with bacilli and is in an early stage of diffuse necrotic change.

SUMMARY.

Acute disseminated tuberculosis.

VIRUS—E IV.

(1.)

Animal Inoculated.—Baboon 72.

Baboon 72 was inoculated subcutaneously with 1 mg. of culture of E IV (from original material), and died 144 days afterwards.

Lungs.

There are a few small, retrogressive tubercles consisting of a fibrous stroma overrun with lymphocytes and leucocytes. No tubercle bacilli have been found.

Liver.

There are a few retrogressive tubercles in which no bacilli have been found.

Kidneys.

There is some interstitial infiltration, the origin of which is uncertain. No tubercle bacilli have been found.

Spleen.

Many large tubercles are present. They are extensively caseous and are surrounded with fibroblasts. Giant cells occur at their periphery. No tubercle bacilli have been found.

Inguinal Gland.

There are a few slightly caseous foci but no bacilli have been found.

SUMMARY.

There is evidence of dissemination, but all the lesions appear to be retrogressive. The spleen is the organ most severely affected. No tubercle bacilli have been found.

(2.)

Animal Fed.—Baboon 74.

Baboon 74 was fed with 1 mg. of culture of E IV (from original material), and was killed 448 days afterwards.

Lungs.

No lesions or bacilli have been found.

Liver.

There is one small, suspicious focus. No bacilli have been found in it.

Kidneys and Spleen.

No lesions or bacilli have been found.

Bronchial Gland.

Lesions of a chronic type are present. Bacilli are rare.

SUMMARY.

With the exception of the bronchial gland, which contains a few bacilli, the tissues appear to have completely resisted infection.

(3.)

Animal Inoculated.—Monkey 178.

Monkey 178 was inoculated subcutaneously with 1 mg. of culture of E IV (with original material), and and died 89 days afterwards.

Lungs.

The lungs contain irregular, ramifying areas of consolidation, the centres of which are partially caseous. Tubercle bacilli are very abundant. Leucocytes are numerous in the lesions.

Liver.

The liver contains some large caseous tubercles in which bacilli are numerous, and also many small slightly caseous lesions in which bacilli are scanty. Leucocytes are abundant, particularly in the larger lesions.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The spleen contains large caseous patches in which bacilli are numerous.

Axillary Gland.

The gland is extensively affected, but the amount of necrotic change is rather small in proportion to the large number of bacilli present.

SUMMARY.

Progressive tuberculosis of an acute type.

(4.)

Animal Fed.—Monkey 182.

Monkey 182 was fed with 1 mg. of culture of E IV (from original material), and was killed 392 days afterwards.

Lungs.

The tissue contains several small slightly caseous patches in which a few bacilli have been found.

Liver.

Small tubercles, apparently of a retrogressive character, are present. Bacilli are very rare.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are a few suspicious foci, but no tubercle bacilli have been found.

Bronchial Gland.

There are large fibro-caseous areas, and in all parts of the gland bacilli are very abundant.

SUMMARY.

The bronchial gland contains very large numbers of bacilli. In the lungs, liver, and spleen there is slight evidence of dissemination but bacilli are rare.

(5.)

Animal Inoculated.—Monkey 218.

Monkey 218 was inoculated subcutaneously with 1 mg. of culture of E. IV (from original material), and was killed 95 days afterwards.

Lungs.

There are discrete and extensively caseous tubercles which are evidently of a chronic type. Bacilli are very rare.

Liver.

There are several small caseous tubercles of a chronic type. Bacilli are very rare.

Kidneys.

One small retrogressive tubercle containing no bacilli has been found.

Spleen.

There are many large, discrete, and extensively caseous tubercles in which bacilli are very rare.

Bronchial Gland.

No lesions or bacilli have been found.

SUMMARY

Disseminated tuberculosis of a chronic type. Bacilli very scanty

General Results.

(1) *In Lemurs.*—Feeding with 1 mg. of culture of E II (original material) produced severe disease in Lemur 18; and the same dose of the same culture, subcutaneously inoculated, caused acute tuberculosis in Lemur 16. The same subcutaneous dose of E III caused acute tuberculosis in Lemur 14.

(2) *In Baboons.*—Feeding with 1 mg. of culture of E IV (original material) produced only slight disease in Baboon 74; and the same dose of the same culture, subcutaneously inoculated, caused disseminated tubercles of retrogressive type in Baboon 72.

(3) *In Monkeys.*—Feeding with 1 mg. of culture of E II (original material) produced only slight disease in Monkey 186; and a similar result was obtained in Monkey 182, fed with 1 mg. of culture of E IV (original material). In Monkey 208 feeding with 50 mg. of culture E II (original material) produced disseminated tuberculosis of a chronic type; in Monkey 212, fed with the same dose of culture of E II (from Calf 404), only slight and apparently non-progressive lesions were produced; and in Monkey 202, fed with 60 mg. of culture of E II (original material), very little evidence of tuberculosis was found. Subcutaneous inoculation of ·1 mg. of E II (original material) produced in Monkey 184 disseminated lesions of a mild type; 1 mg. of the same culture produced still milder infection in Monkey 180, but in Monkey 216 the same subcutaneous dose of the same culture caused general dissemination and abundant multiplication of bacilli, with relatively little tissue destruction. Subcutaneous inoculation of 1 mg. of E II (from Calf 408) caused disseminated tuberculosis of a mild type in Monkey 220. The subcutaneous inoculation of 1 mg. of E IV (original material) caused progressive tuberculosis of an acute type in Monkey 178, and disseminated tuberculosis of a chronic type in Monkey 218. The subcutaneous inoculation of 10 mg. of E II (original material) caused progressive, disseminated lesions in Monkey 168.

V.—LESIONS IN SWINE.

VIRUS—E II.

(1.)

Animal Inoculated.—Pig 184.

Pig 184 was inoculated subcutaneously with 10 mg. of culture of E II (from original material), and was killed 268 days afterwards.

Lungs.

The specimen of lung contains several discrete tubercles which are surrounded by fibrous tissue and contain giant cells. The centres of the larger tubercles are completely caseous. In the smaller tubercles oxyphil leucocytes and the remains of these cells are very plentiful. Tubercle bacilli occur in some of the lesions but are very scanty.

Liver.

Large tubercles of the fibro caseous type and smaller lesions, also chronic in character, are present. Bacilli are rare.

Kidneys.

The specimen contains slightly caseous lesions which are overrun with leucocytes and are surrounded by fibroblasts. No bacilli have been found.

Popliteal Gland.

In all parts of the section caseous patches and giant cells are found. Bacilli are scanty.

Spleen.

There are caseous lesions containing giant cells. No bacilli have been found.

SUMMARY.

Disseminated tuberculosis. The lesions appear to be quiescent, and contain very few bacilli.

(2.)

Animal Inoculated.—Pig 186.

Pig 186 was inoculated subcutaneously with 50 mg. of culture of E II (from original material), and was killed, when ill, 174 days afterwards.

Lungs.

The specimen of lung is completely solidified, and exhibits ramifying caseous areas interspersed with plasma cells and fibroblasts. Many of the affected areas are well vascularised. Bacilli are very numerous all over the specimen and are growing everywhere into small groups.

Liver.

There are small, caseous tubercles, showing a tendency to fibroid change. Bacilli are scanty.

Kidneys.

No lesions or bacilli have been found.

Bronchial Gland.

There are diffuse tracts of caseation, containing many giant cells. Bacilli are moderately numerous, but much less numerous than in the lungs.

SUMMARY.

The lungs, though showing a tendency to conservative change, are severely affected and contain very large numbers of bacilli. The liver shows a much milder type of infection.

VIRUS—E III.

Animal Inoculated.—Pig 194.

Pig 194 was inoculated subcutaneously with 10 mg. of culture of E III, and was killed, when ill, 63 days afterwards.

Lungs.

The lungs show large patches of consolidation which are irregularly distributed and are extensively caseous. Multinuclear leucocytes are abundant. Giant cells occur frequently, and there is also a tendency to fibroid charge. Bacilli are distributed in fairly large numbers all over the specimen.

Liver.

There are several small, caseous lesions which contain giant cells and fibroblasts, but have not a well marked boundary zone. Bacilli are scanty.

Kidneys and Spleen.

No lesions or bacilli have been found.

Submaxillary Lymphatic Gland.

Large, irregular, caseous areas are present, and giant cells are frequent in less advanced lesions. Bacilli are rather scanty.

SUMMARY.

Severe infection of the lungs, but with some attempt at conservative change. Other organs are affected less severely.

VIRUS—E IV.

(1.)

Animal Inoculated.—Pig 188.

Pig 188 was inoculated subcutaneously with 10 mg. of culture of E IV (from original material), and was killed 268 days afterwards.

Specimens of lungs, liver, kidneys, spleen, and a precrural gland have been examined. The lesions are identical in character with those found in Pig 184, which was inoculated subcutaneously with culture of E II, the dose and the duration of the experiment being the same. The chronic character of the lesions and the paucity of bacilli are typical features of swine uberculosis. I have examined 338 tissues derived from 84 swine infected with mammalian tubercle bacilli; in only 21 of these tissues were bacilli numerous; in only 49 of the tissues were the lesions progressive in histological characters, and in only 34 of the specimens did the deposits of bacilli appear to be recent. Excluding 63 tissues from 12 of the animals where the experiments terminated in not more than 14 days, bacilli were numerous in only 14 tissues; the lesions showing evidence of progression in 36 tissues, and evidence of recent deposits of bacilli in only 14. In the tissues of these last-mentioned 72 animals, definite tubercles were found in 175 instances, but 72 of these specimens contained no bacilli. In 31 tissues obtained from 7 pigs, killed within 14 days after feeding, deposits of bacilli have only been found, microscopically, in 4 organs, *viz.*, one spleen, one submaxillary lymphatic gland, and two mesenteric glands.

SUMMARY.

Disseminated tuberculosis. The lesions appear to be quiescent, and contain very few bacilli.

(2.)

Animal Inoculated.—Pig 190.

Pig 190 was inoculated subcutaneously with 50 mg. of culture of E IV (from original material), and was killed 175 days afterwards.

No tubercle bacilli have been found in any of the organs examined. In the specimen of kidney no lesions have been found, but each of the other organs examined (lung, liver, spleen, and a mesenteric gland) contain several small tubercles which are centrally caseous, show a well marked fibrous periphery, and are definitely retrogressive in type.

SUMMARY.

Disseminated tubercles of a retrogressive type; no bacilli have been found.

General Results.

E II, in a subcutaneous dose of 10 mg., produced general tuberculosis in a pig, but the lesions appeared to be quiescent and contained very few bacilli. In another pig, inoculated subcutaneously with 50 mg., the lungs were severely affected and contained very large numbers of bacilli.

E III, in a subcutaneous dose of 10 mg., produced about as much disease as 50 mg. of E II, but the experiment was terminated at an earlier period (63 days as compared with 174 days).

E IV, in a subcutaneous dose of 10 mg., produced the same result as the same dose of E II, and in a dose of 50 mg. has not produced more severe disease.

VI.—LESIONS IN GUINEA-PIGS.

VIRUS—E II.

(1.)

Animal Inoculated.—G.P. 1977.

Guinea-pig 1977 was inoculated subcutaneously with ·1 mg. of culture E II (from original material), and died 66 days afterwards.

Lungs.

The lungs are only slightly affected. There are small foci which are nearly solid, contain aggregations of leucocytes and lymphocytes and swollen endothelial cells, and occasionally show a slight amount of caseation. In these areas a few bacilli are present.

Liver.

The liver is permeated with broad, ramifying, caseous tracts in which bacilli are scanty.

Kidneys.

There are no lesions suggestive of tuberculosis, but a few bacilli have been found within a glomerulus.

Spleen.

There are many small, irregular caseous areas. Bacilli are distributed throughout all parts of the tissue, but not in large numbers.

Mesenteric Gland.

The gland contains small foci which are just commencing to caseate. Bacilli are presen in small numbers.

SUMMARY.

Extensive caseation of the liver and fairly extensive disease of the spleen; slight infection of other organs; bacilli scanty.

(2.)

Animal Inoculated.—G.P. 1978.

Guinea-pig 1978 received an intraperitoneal inoculation with ·1 mg. of culture of E II (from original material, and died 40 days afterwards.

Lungs.

The tissue is semi solid. The consolidated areas have an irregular, patchy distribution, are invaded by leucocytes and lymphocytes, and show signs of commencing caseation. Tubercle bacilli are abundant ; in proportion to their numbers they have produced very little tissue destruction.

Liver and Spleen.

The tissues are riddled with small tubercles which are commencing to caseate and are infiltrated with numerous leucocytes. Bacilli are very numerous.

Kidneys.

The kidneys are very slightly affected. A few bacilli have been found within glomeruli, where they have produced slight necrosis and are associated with small collections of multinuclear leucocytes.

Mesenteric Gland.

Bacilli are numerous in all parts of the gland, which is at the commencement of generalised caseation.

SUMMARY.

Fairly advanced and obviously progressive tuberculosis in all the tissues except the kidneys, which are only slightly affected. Bacilli numerous.

(3.)

Animal Inoculated.—G.P. 2213.

Guinea-pig 2213 was inoculated subcutaneously with 0·1 mg. of culture of E II (from original material), and died 77 days afterwards.

Lungs.

Two portions of the lungs have been examined, and in both there are a few small consolidated foci, generally not larger than an alveolus, which contain a few tubercle bacilli.

Liver.

The greater portion of the liver tissue is replaced by ramifying, confluent, caseous tubercles. They contain giant cells, have a broad cellular periphery in which, in addition to leucocytes and lymphocytes, fibroblasts are generally abundant. Tubercle bacilli are scanty.

Kidneys and Mesenteric Gland.

No lesions or bacilli have been found.

SUMMARY.

Extensive caseation of the liver ; slight infection of the lungs ; bacilli scanty.

(4.)

Animal Inoculated.—G.P. 2579.

G.P. 2579 was inoculated intraperitoneally with ·1 mg. of culture of E II (from Calf 404), and died 41 days afterwards.

Lungs.

The lungs are in a condition of partial consolidation. The process appears to be generalised, rather than patchy, in its distribution. Cells fusions are found here and there, but there are no definite tubercles or caseous areas. Bacilli are very abundant, and in many places have grown into groups of colonies, though associated with very little tissue destruction. A few bacilli have been found within blood-vessels.

Liver.

Bacilli are disseminated very abundantly throughout the tissue, and have frequently grown into colonies. They are associated with an infiltration of leucocytes and lymphocytes. Patches of complete caseation are also present, but in these bacilli are scanty. The greater part of the parenchymatous tissue is disintegrated. Bacilli have been found in some of the blood-vessels.

Kidneys.

No tubercles are present, but there is a general infiltration of lymphocytes and leucocytes, with which bacilli are associated in scanty numbers.

Spleen.

There are numerous patches of early caseation and cell fusions. Bacilli are very abundant in all parts of the tissue, including areas showing no histological change.

SUMMARY.

General dissemination and abundant multiplication of bacilli, with a relatively small amount of degenerative tissue change.

VIRUS—E III.

(1.)

Animal Inoculated.—G.P. 2113.

Guinea-pig 2113 was inoculated subcutaneously with 1 mg. of culture of E III, and died 78 days afterwards.

Lungs.

The tissue is infiltrated with lymphocytes and leucocytes and contains many irregularly outlined areas of consolidation. The larger of these are centrally caseous. Tubercle bacilli are present in the lesions but are not very numerous.

Liver.

The tissue has undergone fibro-caseous transformation and is overrun with leucocytes and lymphocytes. Very little normal liver parenchyma is left. Tubercle bacilli occur, but only in small numbers, in many parts of the tissue.

Kidneys.

Caseous tubercles, overrun with leucocytes, are present. Bacilli are more plentiful than in the liver but are not abundant.

Spleen.

The tissue is extensively caseous and contains tubercle bacilli in scanty numbers.

Cervical Gland.

There are large caseous areas in which tubercle bacilli are moderately numerous (a few in every microscopic field).

SUMMARY.

Disseminated tuberculosis, not very acute in type. Bacilli present in all the lesions but not abundant.

(2.)

Animal Inoculated.—G.P. 2115.

Guinea-pig 2115 was inoculated subcutaneously with ·1 mg. of culture of E III, and died 94 days afterwards.

Lungs.

The lungs contain large, ramifying tubercles which are partially caseous, infiltrated with leucocytes, and contain, in the non-caseous areas, a considerable proportion of fibroblasts. Bacilli, though present in all parts of the lesions, are not abundant. The disease, though obviously progressive, is not of a very acute type.

Liver.

Caseation is more advanced than in the lungs, but in the non-caseous areas there is again some indication of an effort at tissue resistance (the presence of large numbers of swollen endothelial cells and fibroblasts, infiltration with lymphocytes and plasma cells as well as leucocytes). Bacilli are rather scanty.

Kidneys.

In the specimen examined no lesions or bacilli have been found.

Spleen.

Diffuse, early caseation is present. Bacilli occur in all the affected areas but are not abundant.

Portal Gland.

The gland is in a condition of diffuse caseation, more advanced than the spleen. Bacilli are moderately numerous.

SUMMARY.

Disseminated tuberculosis, not very acute in type ; bacilli rather scanty.

(3.)

Animal Inoculated.—G.P. 2116.

Guinea-pig 2116 was inoculated intraperitoneally with ·1 mg. of culture of E III, and died 28 days afterwards.

Lungs.

The lungs contain numerous miliary tubercles which are evidently extending by way of the alveolar walls. In many places the tubercles are becoming confluent. The lesions are in an early stage of caseation and contain numerous bacilli.

Liver.

The liver is extensively affected. The lesions ramify in all directions and have no definite boundary zone. Many of them are completely caseous ; others still contain leucocytes and lymphocytes and the remains of these cells. Bacilli are found in all the lesions but are not very numerous.

Kidneys.

A few bacilli are present, but have caused no definite tissue change.

Spleen.

The tissue is permeated with pale areas which have not advanced to caseation but contain numerous giant cells. Bacilli are present in small numbers.

Bronchial Gland

The gland shows early caseation and contains bacilli in rather scanty numbers.

SUMMARY.

Disseminated, progressive tuberculosis. Except in the lungs, bacilli are not very numerous.

VIRUS—E IV.

(1.)

Animal Inoculated.—G.P. 1979.

Guinea-pig 1979 wss inoculated subcutaneously with ·1 mg. of culture of E IV (from original material), and died 62 days afterwards.

Lungs.

No tubercles or bacilli have been found.

Liver.

The liver is in a condition of diffuse fibro-caseous change. Tubercle bacilli are rare.

Kidneys.

No lesions or bacilli have been found.

Bronchial Gland.

The gland is extensively caseous and shows a tendency to fibroid change. Bacilli are scanty.

Spleen.

The spleen is extensively caseous. Tubercle bacilli are remarkably scanty.

SUMMARY.

The scarcity of bacilli and the tendency to fibroid change indicate a relatively mild type of infection.

(2.)

Animal Inoculated.—G.P. 1980.

Guinea-pig 1980 was inoculated intraperitoneally with ·1 mg. of culture of E IV (from original material), and died 30 days afterwards.

Lungs.

Bacilli are distributed in small numbers throughout the lungs but have only produced a small amount of tissue change. They are often found attached to or within endothelial or epithelial cells and are associated with groups of lymphocytes and leucocytes.

Liver.

Bacilli are present in all parts of the liver and are rather more numerous than in the lungs. They have caused partial necrosis of the parenchymatous cells and an infiltration with lymphocytes and leucocytes. Caseous degeneration appears to be just commencing in many of the lesions.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Tubercle bacilli are fairly numerous and have produced many areas of commencing caseation which are infiltrated with leucocytes.

Portal Gland.

The gland is caseous and contains large numbers of bacilli.

SUMMARY.

Bacilli are freely distributed in the lungs and liver but have not produced an acute type of infection.

(3.)

Animal Inoculated.—G.P. 2487.

G.P. 2487 was inoculated subcutaneously with 1 mg. of culture of E IV (from original material), and died 144 days afterwards.

Lungs.

The lungs are in a semi-solid condition, but only show slight patches of caseous or necrotic change. Bacilli are scanty.

Liver.

The liver contains many tubercles which have not a well defined outline and in the majority of cases are only slightly caseous. They contain giant cells and are abundantly infiltrated with leucocytes and lymphocytes. There is also an infiltration of these cells throughout the tissue. Bacilli are scanty.

Kidneys.

There are several small patches of cellular infiltration amongst which bacilli are occasionally found.

Spleen.

Small, slightly small caseous foci are abundant, and contain bacilli in scanty numbers.

Portal Gland.

The gland substance is almost entirely replaced by fibro-caseous material, the [fibrous element preponderating over the caseous. Bacilli are scanty.

SUMMARY.

Disseminated infection of a very chronic type.

General Results.

The tubercle bacilli isolated from the horse reacted upon the guinea-pig in the same way as the mammalian bacilli of human or porcine origin. All the animals showed disseminated disease, but with less incidence upon the kidneys than upon other organs. The three animals inoculated with E IV are examples of a relatively mild type of infection. The animals inoculated with E II and E III showed more extensive disease, particularly in the liver, but it is noticeable that E III, the more virulent of the two viruses, has not produced infections of more severity than E II.

VII.—LESIONS IN FOWLS.

VIRUS—E I.

(1.)

Animal Inoculated.—Fowl 234.

Fowl 234 was inoculated intravenously with 20 mg. of culture of E I, and died 37 days afterwards.

Lungs.

Small groups of bacilli, the majority no larger than serum grown bacilli, occur in all parts of the tissue. They generally occur within cells which have large, pale, oval or rounded nuclei and a large amount of partially disintegrated protoplasm. These cells, which are probably both endothelial and epithelial, form small clusters, and surrounding each cluster there is often a narrow ring of fibroblasts. Many of the bacilli are fragmentary and there is no clear evidence of multiplication.

Liver.

Small numbers of bacilli, generally fragmentary, are found within all parts of the tissue. They frequently occur within parenchymatous cells. There is no tubercle formation and no marked tissue destruction, but in situations where bacilli occur the cell protoplasm is partially disintegrated. The tissue is infiltrated with leucocytes, but these cells do not show a definite tendency to aggregate round the tubercle bacilli.

Kidneys.

No histological changes have been observed. An occasional bacillus has been found amongst the renal tubules.

Spleen.

Throughout the tissue there are many minute patches suggestive of commencing caseation. In these situations groups of bacilli, more or less disintegrated, occur.

SUMMARY.

Bacilli are generally disseminated, but show no signs of multiplication. They have produced minute lesions suggestive of an irritant action and not typical of tuberculosis.

(2.)

Animal Inoculated.—Fowl 490.

Fowl 490 was inoculated intravenously with 10 mg. of dead culture of E I, and was killed 35 days afterwards.

Lungs.

There are many slightly necrotic foci which generally exhibit a fibroid periphery and occasionally contain giant cells. Broken up bacilli are present in these lesions.

Liver.

A few minute semi-necrotic points are present in the liver and occasionally contain one or two bacilli.

Kidneys.

Neither lesions nor bacilli have been found.

Spleen.

Lesions similar to those found in the liver are present but are more numerous.

SUMMARY.

The disseminated bacilli have produced slight irritant effects.

VIRUS—E III.

Animal Inoculated.—Fowl 154.

Fowl 154 was inoculated intravenously with 1 mg. of culture of E III, and was killed 115 days afterwards.

Lungs and Kidneys.

No lesions or bacilli have been found.

Liver.

(1) There are very numerous minute foci which have pale centres containing the remains of parenchymatous cells and are surrounded by lymphocytes and leucocytes and generally by a thin zone of

fibroblasts. Several tubercle bacilli are generally present in the centres of these foci. (2) There is a relatively small number of much larger lesions which are completely caseous in the centre ; external to this material there is an irregular zone of nuclei which stain imperfectly and are more or less fused together ; the periphery of these tubercles consists of a very broad layer of cells which are mainly lymphocytes, though leucocytes and fibroblasts also occur ; tubercle bacilli are very scanty in these lesions. (3) There are large aggregations of lymphocytes, not generally associated with bacilli, both around the blood-vessels and passing in between the lobules.

Spleen.

Bacilli are distributed both singly and in small groups throughout the tissue. The areas where they occur stain faintly but are not definitely caseous. At the periphery of these foci there is an abundant collection of lymphocytes and leucocytes.

SUMMARY.

General dissemination, probably not progressive, in the liver and spleen.

VIRUS—E IV.

(1.)

Animal Inoculated.—Fowl 134.

Fowl 134 was inoculated intravenously with 1 mg. of culture of E IV (from original material), and was killed 98 days afterwards.

Lungs.

There are a few small groups of partially disintegrated bacilli, which appear to have produced very little tissue reaction.

Liver.

Small groups of bacilli are scattered about the tissue and are generally surrounded by lymphocytes and fibroblasts. The bacilli are generally broken up and show no sign of multiplication.

Kidneys.

A few bacilli are present, but do not appear to have produced any tissue reaction.

Spleen.

There are numerous small, badly staining areas which contain fragments of bacilli.

SUMMARY.

Bacilli are present in all the organs in scanty numbers ; they are evidently in process of destruction and have not led to the formation of tubercles.

(2.)

Animal Inoculated.—Fowl 136.

Fowl 136 was inoculated intravenously with 20 mg. of culture of E IV (from original material), and died 59 days afterwards.

Lungs.

The tissue contains numerous minute, necrotic or partially necrotic foci ; many of these have no cellular boundary zone, but some of the larger are surrounded by a definite layer of fibroblasts. In all these foci tubercle bacilli are present, and in many of them they are numerous. They are generally short (·75 to 2 μ) and straight.

Liver.

Throughout the tissue there are numerous little groups of very small bacilli which have caused partial necrosis in the situations where they are present. There are generally some leucocytes and lymphocytes in their neighbourhood, but the amount of tissue reaction is only slight.

Kidneys.

No bacilli and no definite lesions have been found.

Spleen.

Bacilli are scattered abundantly throughout the tissue and have produced slight necrosis in the situations where they occur.

SUMMARY.

Bacilli are generally distributed in the lungs, liver, and spleen but have only produced slight, localised necrosis.

General Results.

The bacilli, inoculated intravenously, have been generally disseminated by the blood-stream and have produced slight irritant effects in the sites where they have been deposited. They showed no signs of multiplication and have not led to the formation of tubercles. Some of the fowls inoculated with bovine bacilli showed lesions which, though retrogressive, were more extensive and more definitely resembled tubercles.

E.—LESIONS PRODUCED BY MAMMALIAN TUBERCLE BACILLI ISOLATED FROM A GNU AND AN ANTELOPE.

LESIONS IN RABBITS.

VIRUS—Z I.

(1.)

Animal Inoculated.—Rabbit 520.

Rabbit 520 was inoculated intraperitoneally with an emulsion of the original material (portal gland of a gnu), and died 181 days afterwards.

Lungs.

The tissue is consolidated and contains large caseous areas. The lesions are confluent and evidently progressive. In many of the caseous areas the bacilli have grown into large masses; bacilli are also fairly numerous in the non-caseous periphery of the lesions. The bacilli vary from about 2 to 6 μ in length, the usual length being about 4 μ; the longer forms occur within the caseous areas and are generally regularly beaded; the majority of the bacilli outside caseous areas are uniformly stained.

Liver.

Small tubercles occur abundantly throughout the tissue. They contain giant cells and show partial fusion but not complete disintegration of the parenchymatous cells; they have not become definitely caseous, and in general, are suggestive of a mild type of infection. Bacilli are rare.

Kidneys.

No lesions or bacilli have been found.

Axillary Gland.

Three-fourths of the area are replaced by caseous material. Bacilli are not very numerous and have not grown into colonies.

SUMMARY.

Advanced disease of the lungs with great multiplication of bacilli; a mild type of disease elsewhere.

(2.)

Animal Inoculated.—Rabbit 758.

Rabbit 758 was inoculated intravenously with ·1 mg. of culture of Z I, and was killed 91 days afterwards.

Lungs.

The condition of the lungs is identical with that of Rabbit 520. Bacilli are equally abundant, and many long forms are found.

Liver.

A few small tubercles, containing giant cells, are present, but no bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

Inguinal Gland.

There are large caseous areas in which bacilli are rather scanty.

SUMMARY.

Severe disease of the lungs, with abundant multiplication of bacilli; a mild type of infection elsewhere

VIRUS—Z III.*

(1.)

Animal Inoculated.—Rabbit 1166

Rabbit 1166 was inoculated intravenously with 0·1 mg. of culture of Z III (an antelope), and died 74 days afterwards.

Lungs.

The lungs are in a condition of advanced caseous pneumonia and contain very large numbers of bacilli. The bacilli are rather longer than the average. They measure from 2 to 5 μ and are generally curved and partially beaded.

Liver.

There are a few small lesions, characterised by aggregations of lymphocytes and the presence of giant cells. Bacilli are scanty.

Kidneys.

There are many tubercles, which are centrally caseous and have a broad cellular periphery. Bacilli are very numerous. In many of the tubercles they are long, as in the lungs, but in one nodule there is a mass of bacilli which are nearly all short, hardly exceeding in length serum grown bacilli.

SUMMARY.

Advanced tuberculosis of the lungs; numerous tubercles in the kidneys; slight infection of the liver. In both the lungs and the kidneys the bacilli have multiplied abundantly.

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* This virus was also tested on the bovine. Calf 510 was killed 96 days after a subcutaneous dose of 50 mg. of culture. The local lesion is typical of the conservative type. Microscopic specimens of lung, liver kidney, spleen, and a lymphatic gland were found to be normal.

(2.)

Animal Inoculated.—Rabbit 1172.

Rabbit 1172 was inoculated subcutaneously with 50 mg. of culture of Z III (an antelope), and was killed 111 days afterwards.

Lungs.

The tissue shows a patchy consolidation with many areas of caseation. The lesions are partially confluent, but there is a tendency for the individual lesions to be surrounded by a cellular zone. Moderately large groups of bacilli occur in some of the caseous sites, but on the whole bacilli are scanty.

Liver.

There are a few small foci of doubtful nature. No bacilli have been found.

Kidneys.

No lesions or bacilli have been found.

SUMMARY.

Slowly progressive tuberculosis of the lungs.

General Results.

The viruses Z I and Z III have produced in the rabbit advanced, slowly progressive disease identical in type with that caused by other mammalian viruses possessing relatively low virulence for the bovine.

Calf 510, inoculated subcutaneously with 50 mg. of culture of Z III, showed no signs of dissemination in the specimens examined.

ADDITIONAL HISTOLOGICAL DETAILS ILLUSTRATING VARIOUS PHASES OF INFECTIONS WITH MAMMALIAN BACILLI.

I.—THE PROCESSES OF BACTERIAL INVASION AND GROWTH.

A.—EARLY DISSEMINATION.

First Week.

ONE DAY.

Calf 438.—Killed 24 hours after subcutaneous inoculation on the left side of the neck with 44 mg. of highly virulent bacilli of bovine origin. At the site of inoculation the tissues are much distorted by the mass of the material injected. There are several torn blood capillaries, which suggest the possibility that some of the bacilli may have at once gained access to the circulation. A few bacilli are seen within lymph spaces, and bacilli are often found in close proximity to blood capillaries, though not actually within them. In the prescapular gland on the same side only a few bacilli have been found. Most of them occur at the periphery of the gland, either within the peripheral lymph sinus, in the tissues of the capsule immediately external to this, or a short distance within the cortical lymphoid tissue. No bacilli have been found in sections obtained from other situations, viz.: spleen (three sections each from different parts), lungs, liver, and kidneys (two sections of each organ, each section from different parts), a bronchial, a portal, and a popliteal gland (one section of each).

Pig 144.—Killed 24 hours after subcutaneous inoculation under the skin of the ear with 20 mg. of virulent bacilli of bovine origin. The local lesion, not received for examination, is described by Drs. A. S. and F. Griffith as "a small patch of necrosis in the subcutaneous tissues of the ear." In a cervical gland, removed from the same side of the body, there are numerous small groups of bacilli, which are generally distributed both in the lymphoid tissue and in the lymph channels. No bacilli have been found in sections of the lungs, liver, kidneys, spleen, and a bronchial gland (three sections of each organ).

Note.—These observations are recorded with the object of throwing some light on the quantity of bacilli distributed, negative results in the case of any particular tissue being an indication that the amount of dissemination in that tissue is not, at all events, likely to have been great. But from the absence of bacilli in the small bulk of material contained in a histological section it is impossible to infer their complete absence in the tissue as a whole. And, in fact, Drs. A. S. and F. Griffith have shown by inoculation tests (App. to 2nd Int. Rep., Vol. III., p. 237) that for eight different species of animals, including the calf and the pig, general dissemination takes place within 24 hours after subcutaneous inoculation. Thus in Calf 438 they have demonstrated the presence of bacilli in the blood, lungs, spleen, liver, kidneys, marrow, muscle, and a popliteal gland; and in Pig 144 bacilli have been shown to be present in the blood, lungs, and spleen.

THREE DAYS.

Calf 284.—Killed three days after subcutaneous inoculation on the side of the neck with 50 mg. of highly virulent bacilli of bovine origin. In the local lesion tubercle bacilli are readily demonstrable entering lymphatic channels, entering blood-capillaries, within lymphatic vessels, and within blood capillaries. In the prescapular gland on the same side tubercle bacilli are readily found, sometimes occurring singly and sometimes in small groups, though the total number present is not very large. The bacilli occur chiefly in the cortical lymphatic tissue; one has been found within a blood-vessel in the substance of the gland. In a prepectoral gland bacilli are not very numerous, but a few small clumps have been found in the cortex. In one specimen of lung bacilli are very rare, but they have been found within capillaries; in another specimen none have been found. Bacilli have been found in two specimens of liver but are rare. A few have also been found in a bronchial gland. None have been found in the kidneys (four sections), spleen, a mediastinal gland, a portal gland, a mesenteric gland, and a popliteal gland (three sections of each).

Calf 873.—Killed three days after subcutaneous inoculation on the side of the neck with 50 mg. of a virus of human origin known to be only slightly virulent for the bovine. In the local lesion bacilli are readily demonstrable in the same situations as in the local lesion of Calf 284. In the prescapular gland on the same side bacilli are numerous in the cortical substance, in the peripheral lymph channel, and along the course of the lymph channels permeating the cortical substance. In one specimen of lung a single bacillus has been found within a blood-vessel immediately beneath the pleura; in the other specimen of lung examined none have been found. In the liver a few bacilli have been found; they occur within blood-vessels. In a renal lymphatic gland one bacillus has been found near the peripheral lymph sinus. None have been found in the kidneys (four sections), spleen, a deep cervical gland, a bronchial gland, a mediastinal gland, a portal gland, a mesenteric gland, and a popliteal gland (three sections of each). By means of guinea-pig inoculations Dr. Cobbett has demonstrated the presence of bacilli in the spleen, the rib marrow, and a popliteal gland.

Pig 146.—Killed three days after subcutaneous inoculation under the skin of the ear with 20 mg. of virulent bacilli of bovine origin. The local lesion, not received for examination, is described by Drs. A. S. and F. Griffith as "a yellow patch of necrosis, larger than in Pig 144, undergoing softening." In a cervical gland, removed from the same side of the body, bacilli are much more numerous than in the corresponding gland of Pig 144; they are frequent in the lymph channels, abundant in the lymphoid tissue, and occasionally occur within blood-vessels. No bacilli have been found in sections of the lungs, liver, kidneys, spleen, and a bronchial gland (three sections of each organ). But Drs. A. S. and F. Griffith, by the methods of animal inoculation and culture, have demonstrated the presence of bacilli in the blood, lungs, spleen, liver, muscle, and bone-marrow.

FIVE DAYS.

Pig 123.—Killed five days after feeding with 377 mg. of human bacilli (H. 104 E.R.). In the spleen there is a small focus of commencing caseation containing in its centre a clump of bacilli.

Pig 148.—Killed five days after subcutaneous inoculation under the skin of the ear with 20 mg. of virulent bacilli of bovine origin. The local lesion is described by Drs. A. S. and F. Griffith as "a small swelling composed of yellow, necrotic, breaking down substance." In a cervical gland, removed from the same side of the body, bacilli are distinctly more numerous than in the corresponding gland of the three days' animal. In sections of the lungs one small group of four or five bacilli has been found with difficulty. No bacilli have been found in sections of the liver, kidneys, spleen, and a bronchial gland (three sections of each organ). But Drs. A. S. and F. Griffith by animal inoculations have demonstrated the presence of bacilli in the blood, spleen, liver, and muscles.

SIX DAYS.

Rabbit 1544.—Killed six days after subcutaneous inoculation with 50 mg. of bovine bacilli. At the periphery of the local lesion the blood-capillaries are dilated and a few bacilli have been found within them. Evidence of dissemination is also found in the lungs, where there are small, slightly caseous foci containing a few bacilli.

Second Week.

EIGHT DAYS.

Calf 292.—Killed eight days after subcutaneous inoculation on the side of the neck with 50 mg. of highly virulent bacilli of bovine origin. In sections of the local lesion bacilli are readily demonstrable gaining access to the blood and lymph channels. In the prescapular gland on the same side bacilli are very abundant in the cortex, but much less numerous in the medulla; they are also demonstrable in the lymph channels. In a prepectoral gland bacilli are scattered throughout the tissue, but are much less numerous than in the prescapular gland; they often occur in groups, each group consisting of from one to two dozen bacilli. In the lungs there are many small deposits of bacilli, the number in each varying from three or four in the smallest to 100 or 200 in the largest; bacilli are often found within the blood capillaries or attached to the inner surface of the capillary walls. In a bronchial gland bacilli occur in several parts of the cortical lymphoid tissue, though their total number is small. In a mediastinal gland bacilli are more numerous and are present in the lymph sinuses as well as in the gland substance. In each of two specimens of liver bacilli have been found in a few situations, but their total number is very small. In a portal gland bacilli are abundant. There are several small groups of bacilli in the spleen. In a mesenteric gland no bacilli have been found. In a popliteal gland the total number of bacilli is not large, though there are several small deposits, the largest consisting of a group of about 30 or 40; the bacilli are in the lymphoid follicles; none have been found in the lymph sinuses.

Calf 881.—Killed eight days after subcutaneous inoculation on the side of the neck with 50 mg. of a virus of human origin known to be only slightly virulent for the bovine. In a section of the local lesion a few bacilli have been found in the walls of a blood capillary, but the tissue is less vascular than in the corresponding specimen of the three days' calf inoculated with the same virus, and the opportunities for bacilli to make their way into neighbouring vessels are fewer. In the prescapular gland on the same side there is an increase in the number of bacilli compared with the corresponding gland of the three days' animal; none have been found in the lymph spaces of the medulla. In a deep cervical gland removed from the same side of the neck as the prescapular, bacilli are distributed throughout in moderately large numbers. In a prepectoral gland bacilli are almost as numerous as in the prescapular gland. No bacilli have been found in any of the sections of lungs, liver, or kidneys (four sections of each organ); but in the lungs a few minute histological lesions suggest that a few bacilli may perhaps have been present previously. A few bacilli have been found in a bronchial gland but none in a mediastinal gland nor in a portal gland. Two bacilli have been found in the spleen. No bacilli have been found in a mesenteric gland, a popliteal gland, and a suprarenal capsule (three sections of each). By inoculating guinea-pigs, Dr. Cobbett has demonstrated bacilli in the blood, marrow, lungs, liver, kidneys, and a popliteal gland.

Pig 142.—Killed eight days after subcutaneous inoculation under the skin of the ear with 20 mg. of a virulent culture of bovine origin. The local lesion is described by Drs. A. S. and F. Griffith as a "caseo-necrotic local tumour." In a cervical gland, removed from the same side of the body, there is a marked increase in the number of bacilli compared with the corresponding gland of the five days' animal; many of these bacilli are no doubt the result of growth subsequent to arrival in the gland, but many of them are identical in characters with serum grown bacilli, and are therefore probably a portion of the inoculated material. In the lungs bacilli are moderately numerous, and are irregularly distributed in all parts of the tissue; the number present in each focus usually varies from three or four to about twenty, but a few larger groups have been found, their expansion being probably due to multiplication after arrival. In a bronchial gland a few bacilli are present. In the liver bacilli are rare, but a few small deposits have been found. No bacilli have been found in the kidneys. Three or four bacilli have been found in the spleen. By animal inoculations and cultures, Drs. A. S. and F. Griffith have shown that bacilli also occur in the blood, muscle, and a precrural gland.

NINE DAYS.

Rat 61 (B).—Died nine days after intraperitoneal inoculation with 10 mg. of a bovine virus. There is no histological evidence that death at this early date was due to tuberculosis. The lungs contain many small groups of bacilli, and throughout the liver bacilli are abundantly distributed in small groups, both in the interstitial tissue and amongst the liver cells. The substance of the kidneys has not been found to be invaded by bacilli, but masses of these organisms are adherent to some parts of the capsules.

FOURTEEN DAYS.

Calf 288.—Killed 14 days after subcutaneous inoculation on the side of the neck with 50 mg. of highly virulent bacilli of bovine origin. In the local lesion there are plenty of blood capillaries, and both within them and entering them tubercle bacilli are to be found. There is also a very large supply of bacilli in close proximity to blood-vessels. In the prescapular gland on the same side, bacilli are very abundant, forming groups visible with a low power throughout the tissue. Multiplication must, of course, be taken into account, as well as increased dissemination. A prepectoral gland is in the same condition as the prescapular gland. In the lungs, tubercles containing bacilli varying in number from a few to 50 or 100 are to be found in almost every microscopic field (low power view). The increase in the number of foci, as compared with the lungs of the corresponding eight days' calf, points to a decided increase of dissemination. In a mediastinal gland bacilli are abundant. In the liver there are numerous small foci, each containing a few bacilli, but compared with the lungs the total number of bacilli is very small; there is, however, a very considerable increase in the number of foci compared with the corresponding eight days' liver. In a portal gland, bacilli, both singly and in groups, are deposited in many parts of the tissue. In the kidneys there are several small lesions, each containing a few bacilli. Bacilli are distributed more or less uniformly throughout the spleen and are fairly numerous. In a mesenteric gland, bacilli are moderately numerous. In the cortical substance of a popliteal gland there are a few small groups of bacilli.

Calf 869.—Killed 14 days after subcutaneous inoculation on the side of the neck with 50 mg. of a virus of human origin known to be only slightly virulent for the bovine. In the local lesion there is marked evidence of conservative change and the opportunities for bacilli gaining access to the relatively few patent blood-vessels which are present in proximity to them are fewer than in the corresponding lesion of the eight days' animal (Calf 881). In the prescapular gland on the same side, and in a prepectoral gland, bacilli are numerous throughout the cortex, and it seems clear that additional bacilli have been drained into these areas since the eighth day. In each of two specimens of lung a few early lesions are present, and in the majority of these there are three or four bacilli; no bacilli have been found within blood-vessels. In a bronchial gland and in a mediastinal gland there are a few small deposits of bacilli. In one specimen of liver a few bacilli have been found within a blood-vessel; in another specimen there is one small tubercle, in which one bacillus has been found. No bacilli have been found in a portal gland. A few bacilli are present in the spleen. No bacilli have been found in the kidneys (four sections), a mesenteric gland, a popliteal gland, and a suprarenal capsule (three sections of each). By inoculating guinea-pigs, Dr. Cobbett has shown that, in addition to other situations, bacilli were present in the kidneys, a popliteal gland, and the rib marrow; but a guinea-pig inoculated with blood failed to develop tuberculosis.

Pig 150.—Killed 14 days after subcutaneous inoculation under the skin of the ear with 20 mg. of a virulent virus of bovine origin. The local lesion is described by Drs. A. S. and F. Griffith as "a caseo-necrotic tumour with a cavity in the centre communicating externally through a small opening." In a cervical gland, removed from the same side of the body, the deposits of bacilli are very abundant, showing an increase on the corresponding eight days' specimen. In the lungs, deposits of bacilli are moderately numerous, not more numerous than in the corresponding eight days' specimens. In a bronchial gland there are several small groups of bacilli, the deposits being more numerous than in the corresponding eight days' specimen. In the liver deposits of bacilli are scanty in number, showing no definite increase compared with the number found in the corresponding eight days' specimens. In the portal gland there are several early lesions, in most of which one or two bacilli are present. No bacilli have been found in the kidneys (three sections). In the spleen very few bacilli have been found, no more than in the corresponding eight days' specimen. In a mesenteric glaud one bacillus has been found after searching three sections. Drs. A. S. and F. Griffith failed to demonstrate tubercle bacilli in the blood (three culture tubes and one guinea-pig inoculated) and in the marrow (two culture tubes inoculated).

Rabbit 1546.—Killed 14 days after subcutaneous inoculation with 50 mg. of virulent bovine bacilli. A few lesions and bacilli have been found in the liver. There are also suspicious foci in the lungs and spleen, but no bacilli have been found in them.

Third Week.

SIXTEEN DAYS.

Calf 436.—Died 16 days after subcutaneous inoculation with 50 mg. of E I, an equine virus of high virulence for the bovine. There are numerous deposits of bacilli in the lungs, liver, and a mediastinal gland, but no bacilli have been found in the sections of the kidneys. Most of the deposits are not of very recent date but have had time to set up partially necrotic, miliary tubercles, though the amount of the disease is not sufficient to account for death.

SEVENTEEN DAYS.

Cat 32.—Died 17 days after subcutaneous inoculation with 1 mg. of bovine bacilli. Throughout the lungs the alveolar walls are swarming with tubercle bacilli. In the liver bacilli are present in all parts of the tissue but are much less numerous than in the lungs. In the kidneys bacilli are generally distributed in large numbers; they are found in the glomeruli as well as in the interstitial tissue between the tubules. In an inguinal gland, situated near the local lesion, bacilli are so abundant that in many places they form large, continuous masses, which have almost completely obliterated the tissue elements. In this animal, therefore, multiplication, as well as dissemination, has been extremely rapid.

Cat 36.—Died 17 days after intraperitoneal inoculation with 1 mg. of bovine bacilli. The lungs contain large numbers of bacilli. In the liver bacilli are even more numerous. A few groups of bacilli have been found in the kidneys. In an ileo-colic gland bacilli are abundant. This case illustrates rapid dissemination of bacilli in the thorax, as well as in the abdomen, after intraperitoneal inoculation.

EIGHTEEN DAYS.

Calf 258.—Died 18 days after subcutaneous inoculation with 50 mg. of a bovine virus. Bacilli are generally disseminated in large numbers and have produced sufficiently extensive disease to account for death, though in the lungs the individual tubercles, which are very numerous, are smaller than in cases which have lived rather longer. In this case, therefore, dissemination in the calf, after subcutaneous inoculation, has been sufficiently abundant to account for death in 18 days.

NINETEEN DAYS.

Calves 244, 254, 376, 933.—All these experiments were terminated 19 days after subcutaneous inoculation of culture.* The first had received 46 mg. of bovine bacilli and was killed when moribund; the second had received 50 mg. of bovine bacilli and was found dead; the third had received 50 mg. of porcine bacilli and was killed when moribund; the fourth had received 10 mg. of human bacilli and was found dead. All four animals illustrate the same point as Calf 258, the dissemination being very extensive and the lesions sufficient to account for death.

TWENTY DAYS.

Calf 569.—Killed, when moribund, 20 days after subcutaneous inoculation with 50 mg. of a virus derived from human sputum. The dissemination of bacilli in the lungs is quite sufficient to account for death. In the liver bacilli are scanty.

TWENTY-ONE DAYS.

Cat 84.—Died 21 days after subcutaneous inoculation of 1 mg. of bovine bacilli. Bacilli are present in very large numbers throughout the tissues and are found in the blood-stream.

Fourth Week.

TWENTY-TWO DAYS.

Cat 76.—Died 22 days after subcutaneous inoculation with ·1 mg. of bovine bacilli. The tissues are in the same condition as those of Cat 84, though the dose was only one-tenth as large.

TWENTY-THREE DAYS.

Calf 125.—Killed, when moribund, 23 days after subcutaneous inoculation with an emulsion of tissue containing about 360 million bacilli derived from a human virus. This is an instance of early generalised dissemination, with very severe incidence on the lungs, and sufficient to cause a moribund condition in 23 days, produced by a tissue emulsion. Compared with the early fatal cases caused by culture inoculations, the dose is small, since 1 mg. of culture contains a much larger number of bacilli than 360 million.

Rat 76.—Died 23 days after intraperitoneal inoculation with 25 mg. of porcine bacilli known to be of high virulence for the bovine. Bacilli are very abundant in all the tissues of the body, and are found within the blood-vessels.

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* In the first experiment the inoculation was intramuscular.

TWENTY-FOUR DAYS.

Monkey 70.—Killed, when very ill, 24 days after the commencement of feeding (for five days) with tuberculous cow's milk rich in bacilli. At the post-mortem examination Dr. A. S. Griffith found "tuberculosis of mesenteric and colic glands, lungs and bronchial glands." The lungs are typical of advanced tubercular pneumonia and contain very large numbers of bacilli. The case is an instance of rapid dissemination to the lungs after feeding.

TWENTY-SEVEN DAYS.

Goat 7.—Killed, when moribund, 27 days after subcutaneous inoculation with an emulsion of tissue containing about 155 million bacilli derived from a human virus of high virulence for bovines. There is a general dissemination of bacilli amongst the tissues, including the mammary gland, with particularly severe incidence on the lungs. But the number of bacilli in each individual lesion is small.

TWENTY-EIGHT DAYS.

Lemur 14.—Died 28 days after subcutaneous inoculation with 1 mg. of equine bacilli (E. III.), known to be highly virulent for the bovine. Bacilli are very abundant in the lungs and spleen, abundant in the liver, and moderately abundant in the kidneys.

Fifth Week.

THIRTY-ONE DAYS.

Dog 31.—Died 31 days after intraperitoneal inoculation with 50 mg. of bacilli derived from a human virus of low virulence for bovines. There is a general and very abundant dissemination of bacilli. Bacilli are frequently found within blood-vessels.

THIRTY-TWO DAYS.

Dog 33.—Died 32 days after intraperitoneal inoculation with 10 mg. of the same material as that employed for Dog 31. There is a general dissemination, similar to that found in Dog 31; the number of bacilli, though very great, is, on the whole, not quite so large as in Dog 31.

THIRTY-FOUR DAYS.

Calf 477.—Killed, when moribund, 34 days after subcutaneous inoculation in the neck with an emulsion of tissue containing about 3,000 million bacilli derived from a human virus known to be of high virulence for the bovine. There is a copious general dissemination, with particularly severe incidence on the lungs. Bacilli are also abundant in the liver and in lymphatic glands distant from the site of inoculation. Tubercles are present in the mammary gland and contain fairly large numbers of bacilli.

Monkey 114.—Died 34 days after subcutaneous inoculation with ·01 mg. of bacilli derived from a bovine virus. The lungs, liver, kidneys, and an axillary gland all show numerous lesions fairly rich in bacilli. It is noteworthy that the dose inoculated was small.

THIRTY-FIVE DAYS.

Calf 132.—Died 35 days after subcutaneous inoculation with an emulsion of tissue containing about one million bacilli derived from a bovine virus. Generalised dissemination, with particularly severe incidence on the lungs.

Monkey 9.—Died 35 days after subcutaneous inoculation with an emulsion of tissue containing bacilli derived from a human virus known to be of low virulence for the bovine. The lungs, a bronchial gland, the liver, and the spleen all contain very large numbers of bacilli. Bacilli are frequently found within blood-vessels.

Sixth Week.

THIRTY-SEVEN DAYS.

Pig 26.—Killed 37 days after feeding with 10,000,000 bovine bacilli. The lungs contain discrete tubercles in which bacilli are present but rare.

THIRTY-EIGHT DAYS.

Pig 24.—Killed 38 days after feeding with 5,000,000 bovine bacilli. The lungs contain discrete tubercles, some of them with advanced caseation. Bacilli are very rare.

THIRTY-NINE DAYS.

Pig 18.—Killed 39 days after feeding with 1,000,000 bovine bacilli. The lungs contain small tubercles in which bacilli are scanty.

FORTY DAYS.

Calf 109.—Died 40 days after intraperitoneal inoculation with an emulsion of tissue, containing about 48,000 bacilli, derived from a human virus known to be of high virulence for the bovine. The lungs contain numerous miliary tubercles which are in an early stage of formation and are rich in bacilli. Throughout the substance of the liver bacilli are distributed in scanty numbers, and have given rise to a large number of small miliary tubercles, the majority of which have commenced to caseate. The kidneys contain numerous early tubercles in which bacilli are present, but not very numerous. In a bronchial gland there are extensive lesions and bacilli are very numerous. Taking into consideration the very small number of bacilli inoculated, dissemination must have taken place with remarkable rapidity.

Seventh Week.

FORTY-FIVE DAYS.

Calf 597.—Killed, when moribund, 45 days after subcutaneous inoculation with 50 mg. of bacilli of human origin found to be of high virulence for the bovine. There is a generalised and abundant dissemination, the feature of particular interest being that numerous caseous tubercles are present in the udder, and are often in close proximity to the milk ducts.

Monkey 142.—Died 45 days after subcutaneous inoculation with ·001 mg. of bovine bacilli. Enormous numbers of bacilli are present in the lungs, a bronchial gland, the liver, and the kidneys.

FORTY-SEVEN DAYS.

Calf 105.—Killed, when moribund, 47 days after subcutaneous inoculation with an emulsion of tissue, containing about 48,000 bacilli, derived from a human virus known to be of high virulence for the bovine. In the lungs the bacilli have produced innumerable miliary tubercles which are becoming confluent; bacilli are also very numerous in a bronchial gland. In the liver and kidneys the amount of dissemination is much less, though considerable. The special interest of the case is the large amount of dissemination in the lungs following subcutaneous inoculation with a very small dose.

FORTY-EIGHT DAYS.

Cow 68.—Killed, when moribund, 48 days after intramammary inoculation with a tissue emulsion estimated to contain 29 million bacilli of bovine origin. In the lungs there is an extensive dissemination of bacilli, which have led to the formation of numerous miliary tubercles. Some of the lesions are evidently very recent, but many others are large, caseous, and point to the arrival of numerous bacilli at an earlier stage in the experiment. The case is quoted as illustrating dissemination of bacilli to the lungs after intramammary inoculation.

B.—OBSTACLES TO DISSEMINATION.

I.—In Local Lesions.

EARLY STAGES.

Calf 438.—24 hours after subcutaneous inoculation with 44 mg. of virulent bovine culture. The lesion is filled with enormous numbers of multinuclear leucocytes, which exhibit a vigorous phagocytic action upon the bacilli. Relatively few bacilli are in close proximity to blood-vessels, and it is evident that their progress is arrested by meeting the stream of emigrating leucocytes. Some of the bacilli, also, are attached to, if not actually within, the fixed tissue cells.

Rabbit 1542.—24 hours after subcutaneous inoculation with 50 mg. of virulent bovine culture. Amongst and surrounding the bacilli there is a very dense mass of leucocytes; there is no sign of vaso-dilation; the number of blood capillaries is scanty, and no bacilli have been found in any of those present. In specimens of lungs, liver, kidneys, and spleen no bacilli have been found.

Rabbit 1572.—24 hours after subcutaneous inoculation with 50 mg. of human bacilli of relatively low virulence. The general condition is the same as in Rabbit 1542. No bacilli have been found within blood-capillaries, but a few have made their way between muscle fibres. In specimens of lungs, liver, kidneys, and spleen no bacilli have been found.

Calf 284.—Three days after subcutaneous inoculation with 50 mg. of virulent bovine culture. It has already been pointed out that this specimen shows bacilli making their way into the blood and lymph channels. In the present connection it is important to observe that it shows a very much larger number of bacilli which are placed in situations distinctly unfavourable for dissemination, *e.g.* (1) amongst necrotic tissues, entangled in nuclear débris, and apparently cut off from the lymph and blood channels, or (2) within or attached to cells. Bacilli are very frequently found within cells, particularly endothelial or young connective tissue cells and large mononuclear cells, some of which may be wandering cells. Except in the breaking down areas, where bacilli are frequently seen clinging to the nuclear remains of leucocytes, oxyphil leucocytes are much less frequently found to contain bacilli than in the preceding specimen.

Calf 873.—Three days after subcutaneous inoculation with 50 mg. of human bacilli possessing low virulence for the bovine. A section passing through a large mass of culture shows that this material is surrounded on all sides by a very dense layer of oxyphil leucocytes. In situations amongst the subcutaneous muscle where bacilli are more loosely distributed and are fewer, it is seen, as in Calf 284, that though some bacilli have found their way within lymph and blood channels, the great majority of them are debarred from passing into the circulation either by being entangled in breaking down cellular material or by being attached to or enclosed within cells. Many of the fixed tissue cells, as well as the wandering cells, contain bacilli. The multinuclear leucocytes present amongst the larger collections of bacilli show very active phagocytosis; at the outskirts of the lesion, where bacilli are loosely scattered in relatively small numbers, they are contained within other types of cells quite as frequently as within leucocytes and the attraction of the leucocytes towards the bacilli is much less evident.

Rabbit 1543.—Three days after subcutaneous inoculation with 50 mg. of virulent bovine culture. The mass of bacilli has expanded further than in the 24 hours' specimens of rabbits' local lesions, but the general condition is the same as that of Rabbit 1542. In specimens of lungs, liver, kidneys, and spleen no bacilli have been found.

Rabbit 1573.—Three days after subcutaneous inoculation with 50 mg. of human bacilli of relatively low virulence. Compared with the 24 hours' specimens of rabbits' local lesions the main difference is that at the periphery of the lesion the blood-capillaries are numerous and dilated, and their endothelial cells are swollen; but no bacilli have been found within blood-vessels, and there is no evidence of dissemination in the specimens of lungs, liver, kidneys, and spleen.

Rabbit 1544.—Six days after subcutaneous inoculation with 50 mg. of virulent bovine bacilli. The barrier of leucocytes, though still very conspicuous, shows signs of breaking down, but external to this there is a wide and compact zone of lymphocytes. The bacilli are more widely distributed than in earlier specimens; the blood capillaries are dilated and occasionally contain bacilli.

Rabbit 1574.—Six days after subcutaneous inoculation with human bacilli of relatively low virulence. The lesion is expanding. At its periphery, both in the loose connective tissue and where it is invading the muscle, there is a large increase of the fixed tissue cells, many of which contain bacilli. No bacilli have been found in blood-vessels, and there is no evidence of dissemination in the specimens of lungs, liver, kidneys, and spleen.

Calf 292.—Eight days after subcutaneous inoculation with 50 mg. of virulent bovine culture. Bacilli are very abundantly present in areas of necrotic tissue. At the periphery of the lesion they are frequently contained within many varieties of cells, including muscle fibres. Oxyphil leucocytes are extremely numerous and are frequently seen to have ingested bacilli.

Calf 881.—Eight days after subcutaneous inoculation with 50 mg. of human bacilli possessing low virulence for the bovine. The mass of bacilli in the interior of the lesion is becoming surrounded with an imperfectly formed fibrous stroma. External to this stroma the muscle is infiltrated, but the number of bacilli diminishes with remarkable rapidity as we pass from the central groups towards the peripheral area of infiltration, and it is clear that dissemination is already becoming restricted by the formation of a cellular zone of demarcation. The number of bacilli within blood or lymph channels, or in close contact with their walls, is small.

Rabbit 1545.—Eight days after subcutaneous inoculation with 50 mg. of virulent bovine bacilli. The central portion of the lesion has liquefied. In the outlying portions the chief change noted is an increase in the number of plasma cells. No evidence of dissemination has been found in the specimens of lungs, liver, kidneys, and spleen.

Rabbit 1575.—Eight days after subcutaneous inoculation with 50 mg. of human bacilli of relatively low virulence. The general condition is similar to that of Rabbit 1545.

Calf 288.—Fourteen days after subcutaneous inoculation with 50 mg. of virulent bovine culture. The tissue is extensively necrotic and is swarming with bacilli. Though the bacilli are evidently gaining the upper hand and are gaining access to the numerous capillaries which are still present, there is abundant phagocytosis and a considerable amount of newly formed fibrous tissue. The latter, however, is too loosely and irregularly constructed to offer a substantial barrier to the progress of the bacilli.

Calf 869.—Fourteen days after subcutaneous inoculation with 50 mg. of human bacilli possessing low virulence for the bovine. Compared with the eight days' specimen of the animal (Calf 881) inoculated with the same virus, the formation of a fibrous limitation zone is much more advanced and the facilities for further dissemination of the bacilli are very considerably diminished. Only a few bacilli have gained access to the muscular tissue outside the fibrous demarcation area.

Rabbit 1546.—Fourteen days after subcutaneous inoculation with 50 mg. of virulent bovine bacilli. The genenal condition is similar to the eight days' local lesions of rabbits, but there is a decrease in the number of bacilli and an increase in the periperal zone of plasma cells.

Rabbit 1576.—Fourteen days after subcutaneous inoculation with 50 mg. of human bacilli of relatively low virulence. The general condition is similar to that of Rabbit 1546. No evidence of dissemination has been found in the specimens of lungs, liver, kidneys, and spleen.

LATER STAGES.

Calf 769.—Died 17 days after subcutaneous inoculation with 50 mg. of human bacilli possessing low virulence for the bovine, death being due to intestinal disturbances not associated with tuberculosis. As in the 14 days' local lesion of Calf 869, there is an abundant formation of fibrous tissue, but in the present instance this tissue is much more loosely arranged and forms a much less effective barrier against the dissemination of bacilli. Bacilli are abundant in all parts of the section and in several places are seen entering, or within, blood-vessels. Probably the lowered resistance due to intercurrent disease has caused the differences noted between this lesion and the lesion of Calf 869.

Calf 767.—Killed 25 days after subcutaneous inoculation with 50 mg. of human bacilli possessing low virulence for the bovine. The wall of the lesion shows large fibro-caseous areas from which nearly all the blood-vessels have disappeared. Bacilli are abundant within the caseous material but are almost completely confined to this situation. Very few are found amongst the cellular infiltration between the muscle fibres external to the fibrous wall of the lesion.

Goat 7.—Killed 27 days after subcutaneous inoculation with an emulsion of tissue containing 155 million bacilli of a human virus known to be highly virulent for the bovine. The tissue, though highly necrotic, still contains numerous patent blood-capillaries. Bacilli are scanty in number and are generally found amongst caseous material or cellular débris; none have been found in proximity to blood-vessels.

Goat 1.—Died 34 days after subcutaneous inoculation with an emulsion of tissue containing about 620 million bacilli of a human virus known to be highly virulent for the bovine. The animal had received two inoculations on previous occasions. This specimen is useful for comparison with the last. The lesion produced by the final inoculation consists of large caseous areas, bounded and intersected by fibrous tissue, and is swarming with bacilli. There are many dilated blood capillaries, and numerous bacilli are often found in close contact with these. At the periphery of the lesion, where we get early infiltration into the muscle, the fibrous tissue appears to have entangled many of the bacilli and prevented them from spreading; but in some places there is early invasion of the bacilli between the muscle fibres. In these peripheral regions the majority of the bacilli are adherent to, or enclosed within, the fixed tissue cells. On the whole, the resistance of the tissues to the bacilli is relatively feeble. Probably the animal's resistance was lowered by the previous inoculations.

Calf 185.—Killed 40 days after subcutaneous inoculation with ·75 cc. of wet bacilli (a broth culture $4\frac{1}{2}$ months old) obtained from a human virus known to be of low virulence for the bovine. The specimen consists of large, almost completely caseous areas surrounded by dense zones of fibrous tissue. Bacilli are present in the caseous areas, sometimes in fairly large numbers, but they seem to have been unable to penetrate the fibrous barrier. But in some of the caseous patches blood-capillaries persist, and bacilli are found in proximity to them.

Cow 63.—Died 45 days after subcutaneous inoculation with an emulsion of tissue containing about 160 million bacilli originating from human sputum. The lesion contains large caseous areas surrounded by a broad fibrous zone infiltrated with leucocytes and lymphocytes. Bacilli are scanty and appear to be cut off from access to the circulation. The special feature of the case is that though the local lesion shows strong evidence of resistance to dissemination, the inoculation proved fatal in 45 days.

Calf 105.—Killed, when moribund, 47 days after subcutaneous inoculation with an emulsion of tissue containing about 48,000 bacilli derived from a human virus of high virulence for bovines. The histological structure of the lesion is similar to that of the previous case (Cow 63), though in some of the caseous areas bacilli are more abundant. It is noteworthy that the dose, though very small, produced sufficient dissemination to cause a fatal termination in 47 days.

Calf 871.—Killed 91 days after subcutaneous inoculation with 50 mg. of a human virus of low virulence for bovines. The local lesion was found to consist of liquid grey material surrounded by a dense fibrous wall. The portion of this wall which has been microscoped, though not so densely calcareous as to necessitate decalcification, consists of a dense fibrous zone surrounding areas of caseo-calcareous material. In the fibrous zone there are several imperfectly formed capillary channels, most of which are empty. Tubercle bacilli are present in this zone, though not numerous; they are also found amongst the caseo-calcareous material, but never occur in large numbers. This case is quoted as a typical example of the result obtained 90 days after inoculation with a virus of low bovine virulence. It illustrates the dense barrier which prevents the central mass of material, now reduced to a liquid state, from gaining access to the circulation.

Calf 510.—Killed 96 days after subcutaneous inoculation with 50 mg. of a virus, derived from an antelope, possessing low virulence for the bovine. The local lesion contained a large central cavity traversed by fibrous cords. The wall of this cavity, separating it from the subcutaneous muscle external to it, consists of a broad and very compact fibrous layer. On the inner side of the fibrous layer there is a mass of cells and cellular débris, amongst which bacilli are very numerous, large numbers of them being intracellular. In the fibrous wall itself and in the muscle external to it, bacilli, with rare exceptions, are absent. One of the fibrous cords traversing the cavity is found to be composed of dense lamellae of fibrous tissue surrounding a central core consisting of blood-vessels almost completely occluded. Interspersed amongst the fibrous layers there are a few tubercle bacilli. This specimen, like the last, illustrates the effective manner in which bacilli, introduced in the local lesion, are prevented from getting into the circulation.

Calf 267.—Killed 111 days after subcutaneous inoculation with 3 gms. of bacilli, killed by heating, derived from a human virus known to be of low virulence for bovines. The local lesion consists of a fibrous framework filled up with very large numbers of leucocytes and lymphocytes, the latter cells being in the majority. A few small slightly caseous patches are present. Very few bacilli are to be found. This case illustrates the effective manner in which the local lesion has prevented a large mass of dead bacilli from becoming disseminated.

Calf 466.—Killed 139 days after cutaneous inoculation of 50 mg. of virulent bovine culture. The skin at the site of inoculation shows chronic caseous tubercles with numerous giant cells. Bacilli are very scanty. The skin seems to have been very effective in preventing most of the bacilli from passing more than a short distance below the surface, but chronic, retrogressive tubercles in which bacilli are very rare have been found in a gluteal gland and the lungs.

II.—In Lymphatic Glands.

EARLY STAGES.

The series of calves and pigs killed at intervals of from one to fourteen days after subcutaneous inoculation have provided me with abundant material for observing in histological sections the function of lymphatic glands in arresting the progress of bacilli which have recently arrived there. From these examples illustrating early dissemination it is shown (App. to 2nd Int. Rep., Vol. IV.) that in addition to glands near the seat of inoculation, glands situated in a remote part of the body receive, or may receive, bacilli at an early date. For the present purpose the main fact to emphasise, a fact which is illustrated by all the early specimens, is that when bacilli arrive at a lymphatic gland their progress is at once stopped by the tissue with which they come into contact; there is extremely little chance for a bacillus to travel without interruption through a lymph gland and out again by the efferent vessels.

On observing the periphery of the cortex, in the earliest stage of invasion of a calf's lymphatic gland, it is found that bacilli are entangled (1) in the connective tissue where the afferent vessels open into the peripheral lymph sinus, (2) in the framework of the peripheral lymph sinus and the cells lining that framework, and (3) amongst the lymphoid cells of the cortex a short distance internal to the peripheral sinus. Similarly, as we follow the fibrous strands which pass into the cortical substance, with a lymph channel on either side of them, we find the bacilli entangled (1) in the substance of these fibrous strands, (2) in the meshes of the lymph sinuses, and (3) in the lymphoid tissue bordering on the sinuses. During the period of early invasion bacilli rarely make their way into the medullary part of the gland and, when found, they are not lying free in the lymph spaces but are entangled in the cells of the medullary cords. In performing its function of detaining the bacilli the lymph gland receives a variable amount of assistance from the finely granular leucocytes. In the glands of the calf, though an increase in the number of these cells is generally observable from the first, the leucocytes do not seem to be in a hurry to interfere. The majority of them may be described as being on duty but not engaged in active work. Deposits of bacilli are very frequently found with no leucocytes near them. But as soon as the bacilli have begun to cause some damage, indicated by a change in the staining properties of the cells to which they are attached, the leucocytes are attracted to the spot and actively engage in phagocytosis. In the pig's lymphatic glands the general processes by which the normal constituents of the gland arrest the bacilli are the same as in the calf, but the leucocytes appear to commence work more quickly. Within 24 hours after bacilli reach the gland, dense groups of leucocytes may be seen surrounding them and vigorously ingesting them.

The main fact to which attention is directed here is the important part played by the lymphatic glands in arresting the early dissemination of tubercle bacilli. It is not to be assumed that the lymph gland is always completely successful in the performance of this function. There is the possibility, for example, that cells in the gland, such as endothelial cells, may, after ingesting bacilli, break loose and get into the circulation at an early date. This consideration will be dealt with in a subsequent section. And, in general, it is to be borne in mind that in acute infections the apparatus of the lymph gland breaks down very quickly. Thus, for example, fourteen days after inoculation bacilli are found gaining access to the blood stream in a prepectoral gland of Calf 288 and a cervical gland of Pig 150.

LATER STAGES.

For the purpose of considering obstacles to dissemination the following are the main histological features of lymphatic glands in later stages of the infective process :—(1) The lesions, in which bacilli may or may not be demonstrable, may consist of caseous or calcareous material which is effectively cut off from the circulation by being completely surrounded by a dense, non-vascular, fibrous wall. (2) The bacilli may be situated in the centre of caseous areas, or within giant cells, and may be separated from any demonstrable vascular or lymph channels by a more or less abundant zone of wandering cells and cells derived from the fixed tissues. (3) The bacilli may be obviously overcoming the resistance of the tissues and gaining access to the circulation. These three types of disease may be conveniently termed, respectively, retrogressive, indolent, and progressive. But it is important to point out that in actual histological work lesions are constantly being found which are intermediate between (1) and (2), or between (2) and (3) ; and in particular it is frequently not merely difficult but quite impossible to decide whether an indolent lesion is likely or not to develop activity in the future.

In considering what evidence there is of lymphatic glands being completely effective in preventing further dissemination of bacilli, one is first reminded of the fact that many strains of bacilli prove to be of low virulence for the calf when inoculated subcutaneously, and the bacilli which find their way into the lymphatic glands are generally imprisoned there in lesions with more or less definitely retrogressive characters. This evidence is corroborated by Dr. Cobbett's anatomical observations. He states (App. to 2nd Int. Rep., Vol. III., p. 218) that out of 136 calves inoculated subcutaneously with viruses known to be of low virulence for the bovine 76, or 55·9 per cent., exhibited no naked eye manifestations of tuberculosis beyond the glands near to the seat of inoculation.

There are also some interesting cases where the glands receiving the lymphatics draining the udder appear to have completely arrested all bacilli disseminated from the udder after intramammary injection, in so far as this result may be inferred from the failure to detect any tubercular lesions in more distant situations. The following examples are worth quoting :—

Cow 3.—Killed 63 days after intramammary inoculation with culture of human bacilli derived from sputum. Histologically I have found in both inoculated quarters extensive tuberculosis of a slowly progressive type, and some extension of the disease to one of the uninoculated quarters. But the nearest lymphatic glands appear to be quite well able to dispose of the few bacilli which they contain, and at the post-mortem examination no evidence was found of dissemination beyond this situation.

Cow 44.—Killed 41 days after intramammary inoculation with an emulsion of tissue containing two million bacilli derived from a bovine virus of high virulence. I have found very extensive and obviously progressive tuberculosis of the inoculated quarters of the udder, with numerous bacilli. But at the post-mortem examination no sign of disease was found beyond the nearest glands.

Cow 500.—Killed, when moribund, 32 days after intramammary inoculation with an emulsion of tuberculous tissue obtained from a spontaneously infected bovine. The udder shows acute tuberculosis of the inoculated quarters, with abundant multiplication of bacilli. In the nearest glands there are numerous deposits of bacilli, but at the post-mortem examination no evidence of dissemination beyond this situation was found. I may remark, however, that the lesions in the lymphatic glands are clearly of an acute type, and if the animal had lived longer a wider dissemination of bacilli would certainly have taken place from this source. Still this does not alter the significance of the fact, particularly interesting when contrasted with the evidence of much wider dissemination usually seen after subcutaneous inoculation with highly virulent bacilli, that after 32 days the bacilli had failed to produce anatomical evidence of having escaped further than the nearest gland.

The above three instances are quoted to illustrate the point under consideration. To prevent misconception, it may be added parenthetically that in several other experiments a much wider dissemination was observed after intramammary inoculation.

A few cases may also be quoted from certain feeding experiments where there is anatomical evidence that the glands draining the alimentary tract completely arrested the dissemination of bacilli.

Calf 21.—Allowed to suck Cow 3 (suffering from mammary tuberculosis caused by bacilli from human sputum) for 62 days ; killed 226 days after the commencement of the experiment. The mesenteric glands which I have examined contain isolated, retrogressive tubercles, in which bacilli are extremely rare. At the post-mortem examination there was no evidence of disseminated disease.

Calf 156.—Killed 36 days after being fed with tuberculous cow's milk (infected with bacilli of bovine origin) estimated to contain five million bacilli. After examining specimens of the mesenteric glands I summarised their histological features as follows :—"Progressive, caseating tuberculosis. Bacilli fairly "numerous. The vascularity of the lesions is note-"worthy." From these characters it appeared to me probable that some bacilli would effect their escape sooner or later, if none had done so already. But at the post-mortem examination Dr. Griffith found no proof of distant dissemination.

Calf 188.—Killed 44 days after feeding with one million highly virulent bovine bacilli contained in milk. In a mesenteric gland which I examined I found tuberculosis of a chronic type, with scanty bacilli and marked evidence of retrogressive change. At the post-mortem examination no evidence of distant dissemination was obtained.

It ought to be added, however, that these last three cases are more or less exceptional. In feeding experiments conducted on a large variety of animals distant dissemination has frequently occurred and has been confirmed by microscopic evidence.

Another point arises concerning the significance of macroscopic evidence, at the post-mortem examination, to the effect that the tuberculosis has been limited to the nearest glands, or to particular glands in different parts of the body, or to other clearly recognisable foci. It not unfrequently happens in the course of searching large numbers of histological specimens, taken from cases of very slight severity, that bacilli, generally very few in number, are found in foci so minute that they could not possibly be detected by the naked eye. Therefore the post-mortem verdict "normal" does not absolutely exclude the possibility that bacilli may be present. Weight is added to this consideration by Dr. Cobbett's experimental evidence. (App. to 2nd Int. Rep., Vol. III., p. 228.) In seven instances he injected into guinea-pigs tissue (spleen or thoracic glands) which was "apparently normal." The tissue was taken from calves which had been inoculated subcutaneously with human bacilli known to possess low virulence for this species of animal. The animals were killed at periods varying from 54 to 114 days after inoculation. With the tissues of three of the calves the test proved negative, but the tissues of the other four calves produced tuberculosis in guinea-pigs. He also obtained positive results in many instances with material, obtained under similar experimental conditions, which, though presenting some abnormality, exhibited no macroscopic tubercles. These facts serve to show that caution must be observed in interpreting a statement that tuberculosis is "limited" to a particular group of glands.

After making allowance for the above considerations, it must be pointed out that the main fact which my histological work on this subject serves to emphasise is that the lymph gland is highly efficacious in restraining the dissemination of bacilli. Reverting to my rough division of the glandular lesions into (1) retrogressive, (2) indolent, and (3) progressive, it may be said that in (1) it seems practically impossible for the bacilli to escape, in (2) it seems to be possible only in the event of the lesion waking up to greater activity, and in (3) though escape is possible, it is possible only for the bacilli which happen to be in particularly favourable situations, and these, in lesions of this type, are generally very few relatively to the enormous number which are left behind.

III.—In the Spleen.

EARLY STAGES.

In experiments on various species of animals Drs. A. S. and F. Griffith have proved, by means of guinea-pig inoculations, that the spleen contains bacilli within 24 hours after subcutaneous inoculation. It has also been shown by Dr. Cobbett that an emulsion of the spleen removed from a calf, three days after subcutaneous inoculation with human bacilli of relatively low virulence, produced tuberculosis in guinea-pigs. Histological methods are less satisfactory. I have examined sections from the spleen of calves and pigs killed at short intervals after subcutaneous inoculation and find that during the first week, and sometimes during the first fortnight, bacilli are very difficult to discover. Frequently no bacilli can be found after the complete search of several specimens, and the few bacilli occasionally met with are not generally associated with any definite histological lesion. These results serve to indicate that, during the first week at least, the number of bacilli arrested by the spleen under these experimental conditions is probably very scanty. Thus, in the spleens of the two calves killed eight days after inoculation, one with virulent and the other with slightly virulent bacilli, a few bacilli are present in several situations but no definite histological changes have been found. But in the spleen of the calf killed 14 days after inoculation with virulent culture, bacilli are fairly well distributed throughout the tissue and are fairly numerous; they have frequently given rise to small patches of commencing necrosis and are sometimes found within giant cells; they are also frequently found within large mononuclear cells in many situations where there are no definite histological lesions. In this specimen, therefore, there is definite evidence that the spleen has begun to arrest the bacilli. Again, in the series of pigs killed at short intervals after subcutaneous inoculation, the spleen of the eight days' animal is the first in which any definite evidence of detention by the tissues has been obtained. In this spleen the distribution of bacilli appears to be irregular; in the first specimens examined very few could be found, but on subsequent examination of fresh specimens they proved to be rather more numerous and are often definitely entangled in cells, some foci being suggestive of commencing giant cell formation. It is also noticeable in this spleen that the oxyphil leucocytes are beginning to assist in the detention of the bacilli. A few of these cells appear to have been definitely attracted to the foci where bacilli are present, and there is a large reserve force of leucocytes in the adjacent parts of the tissue.

There is thus evidence that bacilli which have found their way into the spleen may, after a short time, find themselves unable to escape. But the activity of the spleen in arresting the progress of bacilli does not appear to be greater than that exhibited by any other organ. In studying early dissemination there is no evidence that the spleen is in any way preeminent as a special trap or filter-bed for the collection and arrest of tubercle bacilli. Compared with a lymphatic gland, the spleen, from its histological structure, is less well adapted for the ready detention of bacilli; and it seems probable that many bacilli which reach the spleen may pass through into the general circulation with little or no delay. In this connection it is interesting to compare the spleen with an organ such as the liver, where the blood-stream is confined to closed capillaries. Taking the early dissemination experiments with calves and pigs as a whole, bacilli are found quite as readily in the liver as in the spleen, and during the first week they appear to be found more readily in the former organ than in the latter. The significance of this comparison is increased when it is remembered that the liver is a much larger organ than the spleen. Similarly, in a series of experiments on rabbits, inoculated intravenously, bacilli are rare in the spleen before the fifth day; on this day there is a decided increase in their numbers and, from the absence of lesions associated with them, they appear to be early arrivals; on the tenth day they are greatly increased in number and have produced early tubercles and many giant cells; but by this time the lungs and liver are very extensively affected and contain extremely large numbers of bacilli.

LATER STAGES.

In spleens examined at later periods after inoculation the lesions may, for the purpose of considering obstacles to dissemination, be divided roughly, like the lesions in lymphatic glands, into (1) the retrogressive, (2) the indolent, and (3) the progressive. But owing to the extreme vascularity of the organ and the ready opportunities for bacilli to gain access to the blood-stream without the necessity of penetrating closed walls of capillaries, facilities for the escape of bacilli are greater than in the case of lymph glands, unless the lesions are in condition (1), *i.e.* definitely walled in by fibrous barriers. Again, in condition (3) bacilli are often found not merely within lesions of an obviously progressive character, but also freely scattered over areas not advanced to caseation; and under these circumstances it seems clear that bacilli can escape into the circulation more readily and in larger numbers than when they are located in acutely infected lymphatic glands.

In some species of animals the spleen, relatively to other organs of the same animal, is affected more readily and more extensively than in other animals; this seems to be due mainly to differences in the pathological susceptibility of this particular tissue, rather than to any difference in the physiological capacity of the organ to act as a filter for the capture and retention of tubercle bacilli.

Calves.

The following examples illustrate lesions found in the bovine spleen at later stages after infection.

Calf 871.—Killed 91 days after subcutaneous inoculation with 50 mg. of a human virus possessing low virulence for the bovine. Sections of the spleen show a few minute caseous patches and a few large giant cells, but three sections have been completely searched without the discovery of any bacilli. Dr. Cobbett, however, found "slight tuberculosis" in guinea-pigs killed 53 days after inoculation with this material. This is an example of a mild type of infection in which the number of surviving bacilli is probably very small and the character of the lesions indicates that their chance of escape is small.

Calf 356.—Killed 105 days after subcutaneous inoculation with 50 mg. of porcine bacilli possessing low virulence for the bovine. In the specimen of spleen there are a few small, slightly caseous foci and some imperfectly formed giant cells; no bacilli have been found. Like the last specimen, this is an example of successful resistance on the part of the tissue.

Calf 102.—Killed 150 days after subcutaneous inoculation with an emulsion of tissue containing about 116,000 bacilli of bovine origin. The specimen contains a tubercle which is in an advanced stage of caseation, is surrounded by a considerable amount of fibrous tissue, and contains very few bacilli; the remainder of the section is normal. In this case, also an example of successful resistance, the bacilli belong to a highly virulent bovine virus.

Calf 103.—Killed 130 days after subcutaneous inoculation with an emulsion of tissue estimated to contain 48,000 bacilli derived from a human virus of high virulence for the bovine. The specimen shows a non-vascular, caseous tubercle, surrounded by a well-marked fibrous zone and containing very few bacilli. This example illustrates the same point as the last, the only difference being that the bacilli are of human origin.

Calf 534.—Killed 87 days after subcutaneous inoculation with 50 mg. of a porcine virus (P. xiv) of moderately high virulence for the bovine. Bacilli are scanty and are confined to semi-caseous areas which contain many giant cells and show a formation of fibroblasts at their periphery. This specimen illustrates the resistance offered by the spleen to porcine bacilli which in several experiments have produced fatal infection in the bovine within 90 days.

Calf 361.—Killed 76 days after intravenous inoculation with an emulsion of tissue estimated to contain 41½ million bacilli derived from a human virus of low virulence for the bovine. The spleen contains large caseous tubercles, containing numerous giant cells and bounded by fibroblasts. Bacilli are scanty and are only found in the interior, non-vascular parts of the tubercles. This is a good example of the restriction of bacilli within typical tubercles of the chronic type; it will be noted that the bacilli were of human origin and proved to be of low virulence for the bovine when injected subcutaneously.

Calf 508.—Killed, when moribund, 73 days after subcutaneous inoculation with 50 mg. of an equine virus. The lungs of the animal were very severely affected. In the spleen there are many small, caseous or partially caseous foci; the older tubercles are partially surrounded with fibroblasts and contain fairly numerous bacilli; the earlier tubercles contain many giant cells, less fibrous tissue, and fewer bacilli; bacilli have not been found outside definitely tubercular foci. The lesions appear to be progressive rather than retrogressive, but there is a definite effort on the part of the tissue to limit the dissemination of bacilli.

Calf 947.—Died 72 days after subcutaneous inoculation of 10 mg. of human bacilli possessing high virulence for the bovine. The spleen contains large caseous areas which are evidently spreading in all directions. Bacilli are rather scanty and are confined to the caseous areas. This is an example of the slowly progressive type of lesion.

Calf 319.—Killed, when moribund, 57 days after intravenous inoculation with an emulsion of tissue containing over 100 million bacilli belonging to a human virus of relatively low virulence for the bovine. Bacilli are freely distributed all over the tissue, which for the most part appears normal; but in some places, where they are particularly numerous, they have produced small caseous foci. The bacilli are freely gaining access to the blood-stream, and the evidence of restriction to their dissemination is only slight. As the bacilli have produced relatively little tissue change, in proportion to their numbers, this is an instance where feeble resistance to dissemination must be associated with the apparent (*i.e.* excluding the possible effect of soluble toxins) indifference or tolerance exhibited by the tissue towards the bacilli.

Calf 212.—Died 25 days after subcutaneous inoculation with 50 mg. of a bovine virus. The spleen is riddled with large, ramifying, caseous areas which are evidently spreading and show no signs of a demarcation zone. Bacilli are very numerous in these areas and are also found in the adjacent tissue. Except in so far as they are entangled within caseous material, the bacilli can readily gain access to the blood stream. This is a typical example of the condition of the spleen after subcutaneous inoculation with a large dose of a virulent bovine virus.

Calf 528.—Died 30 days after subcutaneous inoculation with 50 mg. of an equine virus of high virulence for the bovine. The spleen is similar to that of Calf 212.

Calf 1267.—Killed, when moribund, 56 days after subcutaneous inoculation with 50 mg. of a human virus possessing high virulence for the bovine. The lesions are of the same character as those found in the two preceding animals, the only difference being that, corresponding with the longer duration of life, the caseous areas are more extensive and contain larger masses of bacilli.

Anthropoids and Monkeys.

In the anthropoid ape and monkey the spleen is generally affected very severely; there are extensive areas of caseation, and bacilli are freely distributed both in the necrotic material and in regions which have not undergone degenerative change but allow free access to the blood channels. In a smaller number of instances the resistance offered by this organ appears to have been rather greater. The following are examples of the various types of lesions met with:—

Baboon 72.—Died 144 days after subcutaneous inoculation with 1 mg. of an equine virus of relatively low virulence. In the spleen, which appears to be the organ most severely affected, there are many large tubercles. These are extensively caseous, are surrounded by fibroblasts, and contain giant cells at their periphery. No tubercle bacilli have been found. In this case the spleen appears to have been successful in preventing the further dissemination of the bacilli which have arrived there.

Baboon 2.—Killed, when very ill, 96 days after feeding with 1 million bacilli of bovine origin. There are some patches of slight caseation which contain a few giant cells and are infiltrated with leucocytes. Only one tubercle bacillus has been found; it is situated in one of the caseous areas. This case may be compared with the previous one; the lesions are less advanced, probably owing to the arrival of fewer bacilli in the organ.

Monkey 67.—Killed 199 days after subcutaneous inoculation with ·01 mg. of a human culture possessing low virulence for the bovine. The spleen contains large caseous areas of a chronic type, though not definitely retrogressive. Bacilli are confined to these situations and are scanty.

Monkey 71.—Killed 190 days after subcutaneous inoculation with 1 mg. of the same virus as Monkey 67. The condition of the spleen is identical with that of Monkey 67.

Monkey 62.—Killed 72 days after the commencement of a feeding experiment (feeding for 65 days with tuberculous milk of a cow infected with bovine bacilli). The spleen is in very much the same condition as with Monkeys 67 and 71, but perhaps the bacilli are not quite so scanty; they are all confined to caseous, non-vascular areas.

Chimpanzee 24.—Died 107 days after subcutaneous inoculation with a very small dose (estimated as equivalent to ·00001 mg.) of bovine bacilli. The spleen contains large discrete tubercles which are caseous throughout but are not separated from the surrounding tissue by any demarcation area. The lesions do not contain many bacilli. A few bacilli, possibly recent arrivals, have been found in parts of the tissue which are histologically normal.

Chimpanzee 3.—Died 77 days after feeding with 1 mg. of a human virus possessing low virulence for the bovine. The spleen is permeated with irregular, slightly caseous areas, and contains a great many giant cells. Bacilli, though found in all the lesions without difficulty, are not numerous. Most of the giant cells contain a bacillus.

Chimpanzee 1.—Died 50 days after subcutaneous inoculation with 1 mg. of the virus used for Chimpanzee 3. The spleen contains many large areas of advanced caseation. A few giant cells are present. Tubercle bacilli are moderately numerous in the caseous areas, and also occur in other parts of the tissue, apart from histological tubercles.

Lemur 18.—Died 76 days after feeding with 1 mg. of an equine virus possessing relatively low virulence for the bovine. The spleen contains many caseous areas which are not circumscribed. In some of these situations bacilli are numerous; a few bacilli have also been found amongst apparently normal tissue.

Lemur 14.—Died 28 days after subcutaneous inoculation with 1 mg. of an equine virus possessing high virulence for the bovine. The spleen is swarming with bacilli and is in an early stage of diffuse necrotic change.

Monkey 1.—Killed, when moribund, 52 days after subcutaneous inoculation with a tissue emulsion containing bacilli derived from human sputum. Throughout the spleen, which is in an advanced stage of caseation, bacilli are disseminated in extremely large numbers.

Monkey 2.—Died 41 days after subcutaneous inoculation with a tissue emulsion containing bacilli derived from a bovine virus. The spleen is in the same condition as that of Monkey 1.

IV.—In the Bone Marrow.

It has been shown by Drs. Cobbett and Griffith that the bone marrow is very frequently infected, and that bacilli may be found in this tissue at a very early date, even after subcutaneous inoculation. Thus Dr. Cobbett produced general tuberculosis in a guinea-pig inoculated with the rib marrow of a calf killed three days after subcutaneous inoculation of 50 mg. of human bacilli possessing low virulence for the bovine; Drs. A. S. and F. Griffith obtained a culture from the marrow of a calf killed 24 hours after subcutaneous inoculation with 50 mg. of bovine bacilli; and they also obtained a culture from the marrow of a monkey killed 24 hours after subcutaneous inoculation with 20 mg. of bovine bacilli. When investigating a series of rabbits killed at short intervals after intravenous inoculation with virulent cultures, I made smears from the bone marrow and found that after the fifth day there was a rapid and steadily progressive increase in the number of bacilli found in this situation. In another case (a rabbit which died 23 days after intravenous injection with ·1 mg. of highly virulent human bacilli) I examined sections of the marrow removed from a femur and found that bacilli were abundant and had produced early caseous lesions.

These examples serve to illustrate the fact that the bone marrow is one of the situations where tubercle bacilli may accumulate, though, owing to the loose structure of this tissue, they have ready opportunities to escape.

V.—In the Blood and Lymph Channels.

EARLY STAGES.

Obstruction of the Lumen.—Assuming the blood and lymph channels to be perfectly normal to begin with, the first possibility to be considered is that some obstruction in the lumen may arise from particles of the material inoculated. It is probable that experimental inoculations are very frequently attended by obstructions of this nature. No special interest attaches to the embolisms, leading to sudden death, which result from intravenous inoculations with material in an imperfectly divided state. But apart from such accidents it commonly occurs, with successful inoculations, particularly intravenous inoculations, that small clumps of bacilli become jammed in the capillaries and give rise to minute, petechial haemorrhages. This is a very noticeable feature in the lungs of rabbits killed 24 hours after intravenous inoculation with a small dose of carefully emulsified bacilli. And in examining the local lesions of calves subcutaneously inoculated, one occasionally finds within lymph channels or dilated blood capillaries, clumps of bacilli, which though not very large, seem quite large enough to become arrested when they find their way into the normal capillaries of distant organs.

Adhesion to the Endothelium.—This is by far the most important way in which the dissemination of tubercle bacilli is retarded. It is a special characteristic of the tubercle bacillus that it is a sticky organism, and its propensity for sticking to the endothelial walls of the channel in which it happens to be circulating is of the highest significance in studying the development of the disease. It is this propensity of the bacillus, constantly manifested in whatever part of the body it happens to be present, which gives the key to the histogenesis of the tubercle.

Examples showing the earliest stage of this process, *viz.*, the arrest of tubercle bacilli by the cells of the endothelial wall prior to the development of further changes, can be found most readily by examining animals killed a short time after inoculation. Thus I have found numerous examples in the lungs and livers of rabbits killed 24 hours after intravenous inoculation with 1 mg. of bacilli, and in similar experiments with animals killed at later stages It is also interesting to follow the course of the bacilli after subcutaneous inoculation. In the local lesions of calves killed at a short date (1 to 14 days) after subcutaneous inoculation, bacilli are to be found in the endothelial lining of lymph channels and blood-capillaries, but here the majority of bacilli are passing into the endothelial wall from outside. In some places, however, situated at a little distance from the mass of bacilli, capillary channels can be found with no bacilli outside them but containing one or more bacilli definitely attached to the inner aspect of endothelial cells; in these instances the bacilli must first have been conveyed by the blood or lymph stream and then been arrested before they could make good their escape beyond the origin of the local lesion. In the same series of animals bacilli can be found which have been carried by the blood stream into the lungs or liver and have there become attached to the endothelial cells of capillaries or veins. But arrest by the endothelial cell is quickly followed by other changes, and therefore after the first few days, in mild infections, it is only occasionally and accidentally that a histological section happens to pass through a portion of the endothelial wall which has just received a new arrival. Fresh deposits of bacilli within the endothelial cells of vessels also occur in the terminal stages of a fatal infection. They are to be found in calves fatally infected by subcutaneous inoculation, though in these cases the intravascular circulation of bacilli is rarely abundant, even when the tissues contain very large numbers of bacilli; and out of a large number of acute lesions which I have examined only a small proportion of my sections show bacilli within the endothelium or lumen of vessels. In the case of calves where there has been an enormous multiplication of bacilli after intravenous inoculation the occurrence of bacilli in these situations is naturally much more frequent. With more susceptible animals, such as the chimpanzee and monkey, intravascular bacilli are often abundant, even after subcutaneous inoculation, in the terminal stages, and recent arrivals within endothelial cells are frequently found.

LATER STAGES.

Obstruction of the Lumen.—Obstruction of the lumen may arise—(*a*) in previously normal vessels, from the sudden arrival of a mass of bacilli or from the arrival of cellular or caseous material swept into the circulation from breaking down foci, or (*b*) by progressive tubercular changes of the vessels themselves. From whatever cause the obstruction arises, bacilli are apt to accumulate on the side which meets the flow of the blood-stream. Examples of obstructed lumen occasionally occur in the kidney, where a capillary branch within a glomerulus may become blocked by a plug of bacilli. In such cases the glomerulus becomes the starting point for a tubercular focus, whereas in the absence of any gross obstruction I have rarely found bacilli detained within glomeruli in early cases; the earliest invasions of bacilli are usually found in the interstitial tissue between the tubules. Instances of the cumulative effect resulting from an obstructed vessel are sometimes seen in rabbits inoculated with dead bacilli. In the lungs of one of these animals (Rabbit 488) some of the alveoli contain groups of bacilli which are far too large to have passed through the capillaries and can only be accounted for by supposing that they have accumulated round a small initial obstruction.

Retention by the Endothelial Wall.—Very soon after a bacillus has become attached to an endothelial cell one of two things usually happens; either the cell breaks loose and is carried along in the circulation together with the contained bacillus, or tissue changes take place in immediate proximity to the endothelial cell and tend to circumscribe the bacillus *in situ*. These changes *in situ* may occur either on the internal or on the external side of the endothelium; or probably it is more correct to say that some changes always occur on both sides. The point to which I wish to direct attention now is that as the changes progress the reactive mechanism forms a cell barrier which either (1) projects internally into the lumen or (2) extends externally towards the surrounding tissue.

(1) In the former case we have the formation of a tubercle on the inner aspect of the vessel wall. This is much the rarer event of the two, because the bacilli tend to grow away from the blood-stream, which is an unfavourable medium, and hence the barrier to their progress is usually produced on the external side. Consequently, intravascular tubercles are not often met with except in advanced infection, accompanied by a marked lowering of the animal's resisting powers. The following are examples of intravascular tubercle formation.

Rabbit 30.—Killed 15 days after intravenous inoculation with 1 mg. of bovine bacilli. In the wall of an artery present in the lungs there is a small bulging of the endothelial wall, about three cells in thickness. This projection consists of cells of endothelial type and contains several bacilli. The tissue of the artery external to the endothelial layer is unbroken and contains no bacilli.

Chimpanzee 14.—Died 63 days after feeding with 1 mg. of bovine culture. In the lungs of this animal there are several tubercles within vessels, the outer walls of which are normal. One of the tubercles is extensively caseous and contains numerous bacilli; in another place there is a tubercle in a very early stage of formation, consisting of no more than a heaping up of a few endothelial cells, amongst which a few bacilli are entangled; bacilli are also found in some situations within the endothelial cells of small arteries, but have not led to tubercle formation.

Monkey 2.—Died 41 days after subcutaneous inoculation with a tissue emulsion containing bovine bacilli. In the lungs of this animal bacilli frequently occur within the endothelial cells of blood-vessels. The cells containing bacilli are generally swollen and project into the lumen but seem more likely to become detached and be carried into the circulation than to develop into tubercles. In the liver of the same animal the same condition is observable and sometimes a small group of endothelial cells, containing bacilli, is found projecting into the lumen but still in continuity with the endothelial wall.

Monkey 59.—Killed 79 days after feeding with 10 mg. of culture from a human virus of high virulence for the bovine. Several tubercles are present in the vessels of the lungs; one projects half way across the lumen and contains several bacilli; another, rather smaller, is caseous in the centre and contains many bacilli; and in a third, still smaller and of more recent formation, only one bacillus has been found.

Monkey 65.—Died 80 days after feeding with 5 million bacilli contained in the milk of a cow infected with a highly virulent human virus. The lungs show bacilli (1) within the endothelial cells of small arteries and (2) within small projecting tubercles developed from the endothelial lining of the arterial wall.

Pig 94.—Killed 61 days after subcutaneous inoculation with 1 mg. of bovine bacilli. In some of the veins of the liver tubercles have been found projecting half way across the lumen; they are partially caseous and contain several bacilli.

Calf 121.—Killed, when ill, 36 days after subcutaneous inoculation with an emulsion of human tissue estimated to contain over 360 million bacilli. Attached to a chorda tendinea of the right ventricle of the heart there is a small, completely circumscribed tubercle in which several bacilli are present.

Calf 244.—Killed, when moribund, 19 days after intramuscular inoculation with 46 mg. of bovine bacilli. Attached to a chorda tendinea of the right ventricle of the heart there is a tubercle similar to that found in Calf 121.

Calf 399.—Killed, when moribund, 34 days after intravenous inoculation with an emulsion of tissue estimated to contain over 700 million bacilli derived from a human virus of low virulence for the bovine when inoculated subcutaneously. Projecting on a valve of the right auricle of the heart there is a small tubercle, about the area of the microscopic field (low power). The tubercle is completely circumscribed by a layer of flattened endothelial cells; its interior contains a large number of lymphocytes and shows some patches of commencing caseation; bacilli are present in moderate numbers.

(2) In the ordinary sequence of events, bacilli which have been arrested by an endothelial cell endeavour to escape by multiplying in a direction away from the blood-stream, and hence lead to the formation of lesions, gradually developing into definite tubercles, which have their base on the endothelial wall and project into the surrounding tissue. This process is observable at the commencement of tubercle formation in all parts of the body. As the subject will be dealt with in later sections, when I consider the development of the tubercle, detailed examples illustrating the cellular changes which take place need not be given here. The main fact now requiring emphasis is that when tubercle bacilli have escaped as far as the external side of an endothelial wall they have by no means gained their freedom; on the contrary, they are at once confronted by a barrier formed by the accession of fresh cells, due partly to the increase of the fixed tissue elements and partly to the arrival of wandering cells. As a few special instances illustrating the arrest of the tubercle bacillus on the external aspect of an endothelial wall, the following examples from the endocardium may be quoted.

Calf 361.—Killed 76 days after intravenous inoculation with an emulsion of tissue, estimated to contain 41½ million bacilli, derived from a human virus of low virulence for the bovine when subcutaneously inoculated. On the endothelial aspect of the right auricle there is a small focus, about the area of the microscopic field (low power). A small portion of this focus projects as a flattened convexity outside the level of the cardiac endothelium; the rest, nearly circular in outline, is immediately below the level of the endothelium and has caused a displacement of the cardiac muscle. The focus is a typical tubercle of the chronic type, containing giant cells and scanty numbers of bacilli.

Calf 967.—Killed, when moribund, 48 days after subcutaneous inoculation with 10 mg. of a human virus possessing high virulence for the bovine. In the left ventricle there is a caseous tubercle bordering upon, and slightly projecting beyond the level of, the endothelium but imbedded for the greater part in the muscular tissue. Bacilli are present within the tubercle but are scanty. The tubercle is too far advanced to form an opinion with certainty as to the precise site of the primary focus; the case is quoted here because it appears probable that the lesion has been caused by bacilli endeavouring to pass inwards from the endothelial wall. This opinion is supported by the fact that no other lesions have been found, either microscopically or at the post-mortem, in the substance of the cardiac muscle.

Calf 312.—Killed, when apparently in good health, 90 days after subcutaneous inoculation with 50 mg. of a porcine virus found in other experiments to be of relatively low virulence for the bovine. Immediately beneath the endocardium of the right ventricle there is a narrow tract of infiltration, with cells which are chiefly lymphocytes, extending for some distance under the endothelial layer but only penetrating a slight distance into the muscle. The lesion contains a few tubercle bacilli and is evidently a tubercle of the retrogressive, "small-celled" type.

Calf 399.—A tubercle on an auricular valve of this animal has been mentioned above. In the auricle itself, a small tubercle, containing moderately numerous bacilli, has been found immediately beneath the endothelium and passing into the muscle substance.

VI.—In the tissues external to lymph or blood channels.

EARLY STAGES.

When sections of organs containing early lesions are examined with a view to observing the way in which the progress of the tubercle bacillus is arrested by cells other than those of the endothelial channels by which it has travelled, it is found that there is an intermediate stage, the details of which are difficult to make out, between (1) the first stage, where detention of the bacilli is effected entirely or mainly by the cells in immediate relationship to a recognisable endothelial wall and (3) the later stage, where the channel by which the bacilli have arrived is no longer recognisable and the organisms are present within the tissues, surrounded by an arrangement of cells possessing the characters of an early tubercle. In this intermediate stage (2), endothelial cells are recognisable in association with the bacilli, but they are swollen and more or less distorted from their normal appearance; other cells are also present and appear equally concerned in the process of arresting the bacilli, *e.g.* parenchymatous cells, which generally exhibit some degenerative change of their protoplasm, connective tissue cells, leucocytes, lymphocytes, and large multinuclear cells, which are sometimes situated in the midst of breaking down parenchymatous cells. In short, the general characteristic of these intermediate foci is confusion. Their significance is threefold. They illustrate (*a*) the continued importance of the endothelial cell in this intermediate stage which is preparatory to the formation of a tubercle, (*b*) the fact that all the cells which form the environment of the bacilli are confused or disorganised, and (*c*) the fact that all the cells present help, in one way or another, to prevent the dissemination of the bacilli, *e.g.* by phagocytosis, by disintegration, or by simply collecting as a barrier between the bacilli and the normal tissue. A special word may be said about (*b*) the element of confusion. In this intermediate stage the efforts of the tissue to localise the bacilli are not systematic; there is no concerted action first of one particular type of cell, and then of another, then the metamorphosis of one type of cell into something quite different, the disappearance or disintegration of another type, and so forth; on the contrary, at this stage of the process all the cells engaged are supremely indifferent to any and every theory of tubercle formation; their disposition and their relationship to the bacilli are simply expressive of disorganisation.

In the lungs there is a serous exudation from the capillaries, causing a thickening of the alveolar walls and a swelling up of the cells of the alveolar epithelium; the endothelial cells are enlarged, increased in number, irregularly placed, and severed from their normal relations to a capillary channel; oxyphil leucocytes are present, but in some animals, such as the calf, are distributed irregularly and do not appear to be very actively engaged in phagocytosis, whilst in other animals, such as the pig, they have converged upon the bacilli in large numbers even at this early stage and are actively phagocytic; small lymphocytes are also present, but many of them appear to be wandering aimlessly about, unaware that their existence is imperilled by close proximity to active tubercle bacilli. This is the confusion of cells amongst which the bacilli are detained when they first encounter the tissues outside the endothelial wall of a lung capillary.

In the liver there is less evidence of a serous exudate in the intralobular foci; these consist of partially disintegrated liver cells, endothelial cells, multinuclear cells or cell-fusions, and generally a few lymphocytes and leucocytes, the general picture indicating reaction to an irritant, but no systematic process of repair. When the bacilli occur in the interlobular connective tissue the general swelling up of the fixed tissue cells indicates that there has been a considerable amount of exudation, and the wandering cells, both lymphocytes and leucocytes, are attracted to the foci in large numbers, the lymphocytes generally being in the majority, except in animals where the leucocytes are particularly active; but the bacilli, with the exception of such as may be contained within leucocytes, are generally found entangled amongst cells of connective tissue or endothelial type.

In the kidneys the bacilli are generally found between the renal tubules in the cortex; they rarely occur within the glomeruli, and as there is no evidence that they are excreted in the glomeruli and then absorbed by the renal cells, it appears probable that they reach the intertubular tissue by way of the various capillaries emerging from the glomeruli. The fixed and wandering cells in this interstitial tissue play the first part in their detention.

LATER STAGES.

The later stages of the process vary according to the severity of the infection induced. In situations where the bacilli are of high virulence and numerous, the earlier stage, characterised as one of confusion, is continued into one of progressive and diffuse necrotic change with no peripheral demarcation zone; the cell details of such lesions, before the necrosis is too advanced to obscure them, exhibit, like those in the earlier stage, an entire absence of system or structure and a complete indifference towards any of the better accredited theories of tubercle formation. Under such conditions the efforts of the tissue to restrain the advance of the bacilli are obviously ineffective, and dissemination progresses both by direct extension of the affected areas and by escape of the bacilli into the circulatory channels. At the other extreme of the scale there are examples of completely successful tissue resistance, where the bacilli, or the foci which previously contained bacilli, are comprised in a central nucleus of caseo-calcareous material, completely cut off from the circulation, and surrounded by a dense fibrous zone. Intermediate between these extremes, examples are constantly met with where the detention of the bacilli is of a more or less temporary character; the bacilli may be contained within epithelial cells, or within endothelial cells, connective tissue cells, or leucocytes; they may be surrounded by a zone of wandering cells, or be partially cut off from the blood and lymph channels by a more or less effective barrier of fibroblasts.

C.—THE PROGRESSIVE ESCAPE OF BACILLI.

I.—By Simple Extension of Tubercular Foci.

When a diseased focus expands, the area over which the bacilli are distributed is concomitantly extended. This condition requires no explanatory discussion.

II.—By Increased Vascularity.

Unless the bacilli are situated in definitely retrogressive lesions and are completely cut off from the blood-stream, their escape may be facilitated by an increased vascularity. Blood capillaries are very frequently present in the midst of breaking down tissue where bacilli occur, and their walls are generally weakened by the degenerative process; when distended, these walls are more liable to give way than those of normal capillaries; and thus the entrance of bacilli is made easier. This is a possible danger in the treatment of tuberculosis by inducing hyperaemia.

The following examples illustrate various points concerning the incidence of increased vascularity in tuberculosis.

EFFECT OF TUBERCULIN.

Calf 135 was killed 159 days after subcutaneous inoculation on the left side of the neck with a small dose, estimated at about 13,000 bacilli, of a human virus possessing low virulence for the bovine. Tuberculin was inoculated, 19 hours before death, on the right side of the neck and produced a rise of temperature amounting to 2·4° C. In the right prescapular gland the blood-vessels are greatly dilated and the entire gland is engorged with red corpuscles. These corpuscles occur all over the gland, filling many of the lymph sinuses and overflowing into the lymphoid nodules, the engorgement being greatest in the medullary part of the gland. The gland contains neither tubercle bacilli nor lesions suggestive of tuberculosis. It is evident that if any bacilli had been present their opportunities of gaining access to the circulation would have been greatly increased by the hyperaemia caused by the tuberculin.

HYPERAEMIA OF LOCAL LESIONS.

After bacilli have been inoculated subcutaneously there is always a local increase of vascularity. This, as I have shown in the case of calves, persists for a variable length of time, dependent upon the susceptibility of the animal for the bacilli introduced. With bacilli of high virulence for the calf the hyperaemia is maintained for a considerable period, but with bacilli of low virulence there is a marked diminution of vascularity during the second week after inoculation, and this diminution, as found by examining lesions removed at later periods, is progressive. It is obvious that the hyperaemia at the site of inoculation is an important factor in increasing the dissemination of bacilli.

HYPERAEMIA OF TUBERCULAR LYMPHATIC GLANDS.

Hyperaemia may be induced in a lymphatic gland by various causes. It may be due to the absorption of irritant material derived from tubercle bacilli, as is shown in the effect produced by tuberculin, or to other irritant matter drained from a caseating focus, or to the reception of various irritants not associated with the tubercle bacillus. When tubercle bacilli are already present in a gland, it is important to remember that hyperaemia, arising from any of these causes, may facilitate their dissemination, or, as it is sometimes termed, "light up" the infection. In the tubercular glands which I have examined, vascular engorgement is frequently found. But it is worth noting that this condition is by no means either a necessary preliminary, prior to the reception of tubercle bacilli, or a necessary concomitant of early deposits of these organisms. Glands removed at short periods after the commencement of the experiment often fail to show hyperaemia, although they contain deposits of bacilli, and although they may contain clear evidence of pathological changes in the neighbourhood of these deposits. Statements are often copied from one text book into another to the effect that in tuberculosis of the lymph glands we have first a preliminary stage of hyperaemia; then, concomitantly with the deposit of bacilli and the formation of tubercles, a diminution of vascularity; and finally, a complete obliteration of all blood-vessels from the tubercular foci. My experience does not add support to general statements of this nature. It is true that hyperaemia may be present at the commencement of infection, but it is also true that it may be absent; and although advanced tubercles are frequently non-vascular, this is by no means a general rule; on the contrary, glands presenting advanced lesions often contain patent blood capillaries in intimate contact with the affected areas, and these channels are not infrequently more dilated and more engorged with blood corpuscles than the vessels of a normal gland. To demonstrate this condition it is important to use material hardened in a good fixative for blood corpuscles and to employ paraffin sections fixed on the slide or coverslip (in order to prevent material from falling out of the section).

From an examination of lymphatic glands removed at various stages of infection, I find that increased vascularity may occur at any stage, and that dilated capillaries frequently occur in positions which give tubercle bacilli the opportunity of being carried away by the blood-stream.

HYPERAEMIA OF THE LUNGS.

In all stages of the disease, dilation of the blood capillaries is very commonly found in association with deposits of bacilli. Animals killed at short periods after subcutaneous inoculation afford examples of this condition; but here the congestion is associated with a serous exudation, a swelling up of the endothelium and the alveolar epithelium, and in general with conditions favouring the absorption of bacilli by the tissues *in situ* rather than their progressive escape by the blood-stream. At a late stage in the disease, for example, in animals fatally infected by subcutaneous inoculation of 50 mg. of culture, engorgement of the blood capillaries is again very common, and even in the consolidated areas which have advanced to caseation the vascularity may be still considerable. Under these circumstances, as bacilli are distributed throughout the tissue in large numbers, the vascularity is obviously favourable to their progressive escape. And in animals killed after intravenous inoculation, vascular engorgement of the lungs is also very common and is frequently present in a high degree.

This condition of the lungs is not confined to cases where the bacilli were originally introduced in large or overwhelming doses. It is found, for example, in the lungs of Calf 132, which died from acute miliary tuberculosis 35 days after subcutaneous inoculation, the dose in this case being only one million bacilli.

And in less severe types of the disease, where the tubercles are discrete and vary in severity from the slowly progressive to the indolent or apparently retrogressive, the periphery of the foci is often highly vascular, and blood-vessels are sometimes found in their interior. In these cases bacilli may sometimes be found in proximity to the vessels, though the total number of bacilli present is usually much less than in lesions of the acute type.

HYPERAEMIA OF OTHER ORGANS.

In the case of other organs where tubercle bacilli are present, hyperaemia ought to be mentioned as a possible event assisting in their dissemination. In the liver and kidneys this phenomenon is much less frequently found than in the lungs and lymphatic glands. Histologists who draw a distinction between inflammatory manifestations associated with the presence of tubercle bacilli and the changes directly concerned in the evolution of the tubercle might express this difference by saying that lesions in the lungs and lymph glands are more frequently associated with changes of the inflammatory type. For my own part I think the term inflammation is too vague and comprehensive to form the basis of a useful distinction.

The main fact to which attention is now called is that, largely owing to the more compact structure of organs such as the liver and kidneys, lesions in these situations are not usually associated with a zone of loosely arranged and easily dilated capillaries. But it is true of tubercles in these regions, as it is true of tubercles in most parts of the body, that both in early and in late stages vascular channels may often be present, and access to these is sometimes gained by the bacilli.

Hyperaemia of the cow's mammary gland is of special importance. This organ, when yielding milk, is subject to periodical vascular dilatation ; hence it is particularly liable to receive bacilli carried from distant sources ; and the vascular stream has exceptionally favourable opportunities for washing out bacilli, already lodged in the tissue, in the direction of, and directly into, the alveolar tubules and the milk ducts and sinuses.

III.—By Infection of Proliferating Foci.

Cellular proliferation, with the formation or attempted formation of new vascular and lymph channels, is a constant feature of early lesions. In some cases, where the bacilli are abundant and of very high virulence, this process is soon arrested and gives way to necrotic change ; and in other cases, where the bacilli are scanty and of low virulence, the process is replaced, sooner or later, by fibroid transformation. But between these two classes of extremes there is a very wide margin, comprising a large variety of tubercles in intermediate stages, where the tendency to form new capillary channels is maintained and is particularly in evidence at the margin of the lesions. This feature is very conspicuous in the local lesions of animals killed a few days after subcutaneous inoculation, and also at the periphery of the lesions at later stages, so long as the lesions are in process of expansion. Similarly with tubercles found in all other situations of the body, where expansion is going on and is not accompanied by rapidly diffuse necrotic change, this same tendency to new capillary formation may be observed. Into these new channels tubercle bacilli may find their way, either by direct inclusion when the channel is in process of formation, or by drainage from areas with which the channels are connected. The number of bacilli which actually become disseminated in this manner naturally depends upon the number which are able to gain access to these new channels and upon the capacity of the channels for conducting them. In local lesions where a large mass of bacilli is artificially introduced, and where the inflammatory reaction which follows is accompanied by the abundant production of well formed capillaries completely linked up with the circulation, these channels appear to be responsible for the conveyance of a large number of bacilli. But in ordinary, small, spontaneously formed tubercles, bacilli as a rule, though not without exceptions, are relatively less abundant in the cellular zone ; they are also particularly liable to absorption by the swollen, proliferating cells which are forming the new endothelium ; for these reasons the escape of the bacilli by way of newly formed capillaries is not without difficulty. In histological sections bacilli are sometimes found either actually within such channels or on the point of entering them, but the chance of their being carried very far is less favourable than when they have gained access to an older capillary, with smooth, flattened walls and a dilated lumen. At the same time there can be no doubt that bacilli actually are disseminated in the former way.

The following examples, taken from various experiments on bovines, illustrate the formation of new endothelial channels in proliferating foci and their relation to the dissemination of bacilli. The tubercular new growths on the pleural aspect of the lung and similar formations on the surface of the liver are of particular interest because they are separated from the parenchymatous tissue and therefore afford a good demonstration of the facilities for the conveyance of bacilli by newly formed channels.

Heifer 26.—Killed 115 days after intravenous inoculation with an emulsion of tissue containing bovine bacilli (probably scanty in number). The lungs contain definitely circumscribed and completely isolated tubercles, centrally caseous and surrounded by a fibrous zone. Except in their centres, the nodules are richly supplied with blood-vessels. On the pleural surface, adjoining one of the tubercular nodules, is a tufted outgrowth, consisting of a widely open and irregularly arranged meshwork of delicate fibrils, amongst which are several blood-vessels and a scanty number of cells, the whole being bounded by a single layer of pleural epithelium. This case illustrates an early stage of these vascular outgrowths. Bacilli, in this case, are scanty.

Cow 74.—Died 299 days after intramammary inoculation with an emulsion of the same material as Heifer 26. The inoculated quarters show extensive tuberculosis, with very large numbers of bacilli. In some places, particularly near the nipple, many new capillary channels have been formed ; and the tissue as a whole, except where calcareous or completely caseous, is highly vascular.

Calf 89.—Killed, when very ill, 23 days after intravenous inoculation with a tissue emulsion estimated to contain about 300,000 bacilli. The bacilli were derived from a virus isolated from human sputum. The lungs contain numerous consolidated foci, irregular in outline and highly vascular. In some places, at the margins of a tubercular focus, the walls of blood capillaries which contain red corpuscles are seen to be proliferating, and in more or less complete continuity with these are seen newly formed, empty capillary channels. Bacilli are very numerous.

Calf 93.—Died 73 days after intraperitoneal inoculation with a tissue emulsion, estimated to contain about 600,000 bacilli, from the same material as that inoculated into Calf 89. Projecting from the surface of the lung are prominent outgrowths which are found microscopically to contain a dense network of fibrous tissue, and to be partially necrotic in the centre. The interstices of the fibrous stroma are, particularly in the outer portions of the nodules, occupied by an enormous number of blood channels engorged with red corpuscles. On part of the free surface of the nodules there is, external to the fibrous structure, an outer layer of loose fibrillar tissue containing very few cells and consisting mainly of a widely meshed stroma which stains red in van Gieson preparations. In this stroma, also, engorged blood-vessels are numerous and very conspicuous. Bacilli are very abundant.

Calf 105 and Calf 114.—The former animal was killed when moribund 47 days after subcutaneous inoculation with a tissue emulsion containing about 48,000 bacilli of human origin ; the latter was killed 161 days after subcutaneous inoculation with a tissue emulsion containing about 1,000 bovine bacilli. The livers of both animals exhibit mushroom growths which project from the capsule and are practically identical in the two cases. These projections are connected with the liver substance by a broad, fibro-vascular base ; they are centrally caseous, contain giant cells, and are identical in general characters with ordinary circumscribed tubercles found in other situations ; they contain numerous bacilli.

Calf 141.—Killed 53 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at 250,000, derived from a virulent human virus. On both the lungs and the liver of this animal outgrowths projecting from the surface are present.

Heifer 231.—Killed 109 days after subcutaneous inoculation with a tissue emulsion containing bacilli, estimated at 1,800,000, derived from a human virus of high virulence for the bovine. On the liver there is a flattened, mushroom-like outgrowth attached to the capsule by a narrow pedicle. It contains several giant cells and a few patches of early caseation. Its ground-substance consists of a widely open network of branching connective tissue fibrils This is permeated by a large number of capillary channels, and also contains a few arteries and veins ; many capillaries are seen in process of formation.

Calf 281.—Killed, when ill, 54 days after subcutaneous inoculation with a tissue emulsion containing bacilli, estimated at 11 million, derived from a human virus which was at first of low and afterwards of high virulence for the bovine. The lungs contain numerous tubercles. Some of the older and larger are partially marked off from the surrounding tissue by a layer of fibroblasts; but in the younger tubercles, which are more numerous and quite irregular in outline, this demarcating layer is absent. There is a notable proliferation of endothelial cells in the tubercles, with the formation of new blood capillaries. This is an example taken from a tissue presenting lesions of an intermediate type of severity.

Calf 365.—Killed 61 days after subcutaneous inoculation with an emulsion of human material estimated to contain about 100 million bacilli. The virus was found in other experiments to be of low virulence for the bovine. In the specimen of lung there are numerous small tubercles; they contain many giant cells, and some of the largest are commencing to caseate. They show a well marked proliferation of endothelial cells, with formation of new capillary channels, and many of them are highly vascular. Tubercle bacilli occur within the tubercles but are scanty. This is an example derived from tissues infected with a virus of relatively low virulence, where the bacilli are scanty and not likely to disseminate far along the newly formed channels.

IV.—By Breaking Down Foci gaining Access to the Circulation.

This is a common and important means of progressive dissemination. Its frequency and its significance are often insufficiently appreciated, owing to an implicit acceptance of the doctrine that a tubercle is essentially a non-vascular structure. But such a statement, though true in many cases of the human disease where the lesions are very chronic in character and contain few or no demonstrable bacilli, is not true as a general description of the typical tubercles observed under the more favourable conditions of animal experiment. My histological specimens, obtained from different animals and under different experimental conditions, including infections of the more chronic type, make it clear that in the processes of tissue disintegration blood-vessels and capillaries often survive intact after the surrounding tissue has become completely caseous. This survival is only temporary; the vessel walls must give way before the destructive process sooner or later, and during the period of their disintegration an opportunity may be given for the bacilli in their environment to escape into the circulation. I am not ignoring the important fact that one general feature of the tubercular process is the tendency to produce obliterative changes in the vessel walls; my point is that this process does not always come into operation quickly enough to efficiently block the vascular channels before bacilli can find their way in; and even in completely avascular tubercles, whether in animals or man, there is always the possibility to consider that bacilli may have made their escape from these foci before the vascular obliteration was complete. In an earlier section I considered the obstacles to further dissemination which the bacillus encounters when it arrives at different parts of the body; following the fate of the bacillus a stage further, it may be said in general terms, applicable to foci in any part of the body, that the bacillus may escape from its environment by setting up destructive processes which break the continuity of the vessel walls and allow access to their interior. The following examples are quoted as illustrating this method of dissemination.

LOCAL LESIONS.

It is only necessary to refer to the facts already mentioned, *viz.*, that in early stages, and also in later stages so long as the lesions are progressive, the tissue is highly vascular; during the process of disintegration gaps are constantly being formed in the vessel walls, and through these gaps bacilli may enter the circulation, and can be demonstrated actually in process of entry.

THE SPLEEN AND BONE MARROW.

It has already been pointed out that owing to their loose structure these tissues do not offer such an effective mechanism for the detention of bacilli as more compact organs. It may now be added that, for the same reason, they afford more ready opportunities for bacilli to escape from breaking down foci.

LYMPHATIC GLANDS.

Calf 288.—Killed 14 days after subcutaneous inoculation with 50 mg. of a bovine virus. In a section of a prepectoral gland, which contains numerous lesions commencing to break down, one of these foci has broken into the walls of a dilated blood capillary and bacilli can be traced from the periphery of the lesion into the centre of the vascular channel.

Pig 150.—Killed 14 days after subcutaneous inoculation with 20 mg. of a bovine virus. A section of a cervical gland shows exactly the same condition as that found in the prepectoral gland of Calf 288.

Calf 587.—Died 35 days after subcutaneous inoculation of 50 mg. of a human virus of high virulence for the bovine. In a thoracic gland, which is extensively affected and contains many bacilli, the blood capillaries are numerous and dilated, and in some of these bacilli have been found passing in from the adjacent lesions and situated in the lumen of a vessel. This case is quoted as an example of the condition commonly found in the lymph glands of bovines fatally infected by subcutaneous inoculation.

Cow 4.—Killed 34 days after intramammary inoculation with bovine bacilli. In a supramammary gland which is extensively caseous and highly vascular bacilli are found in several places just within the lumen of capillary channels and evidently passing in from the breaking down tissue at the periphery.

Cow 18.—Killed 35 days after intramammary inoculation with bovine bacilli. The supramammary gland examined is in the same condition as that of Cow 4 and also shows bacilli on the point of entering vascular channels.

Monkey 18.—Killed, when moribund, 17 days after intravenous inoculation with bovine bacilli. A bronchial gland, which is extensively affected and contains very large numbers of bacilli, shows bacilli on the point of entering vascular channels. This case is quoted as an instance of a condition not uncommonly found in the extensively affected lymphatic glands of anthropoids and monkeys. Other examples, not requiring special description, have been found in the glands of Monkey 114 (died 34 days after subcutaneous inoculation with bovine bacilli), Monkey 9 (died 35 days after subcutaneous inoculation with human sputum bacilli), Chimpanzee 3 (died 77 days after feeding with human bacilli), and Monkey 73 (died 89 days after feeding with human bacilli).

Goat 1.—Died 34 days after the last of four subcutaneous inoculations with human viruses. A thoracic gland shows many small patches of early caseation and contains numerous dilated blood capillaries. In a few places, where the vessels border on the caseous areas, bacilli are seen which have just penetrated the capillary wall.

Cow 74.—Died 299 days after intramammary inoculation with bovine bacilli. A special feature of one of the supramammary glands is that it is highly vascular, notwithstanding the long duration of the experiment. The gland contains numerous tubercles of the slowly progressive type, and bacilli are present in large numbers. In several places where patches of early caseation border upon the blood capillaries, bacilli are in close contact with the capillary wall, and in one place I have found bacilli actually within the capillary.

Pig 26.—Killed 37 days after feeding with 10 million bovine bacilli. An ileo-caecal gland contains numerous caseating areas in which bacilli are moderately abundant. Vascular channels are present in definitely affected areas. This fact is interesting as indicating one of the ways in which alimentary tract tuberculosis may become disseminated.

Calves 154 and 156.—Both these animals were killed 36 days after being fed, the former with 10 million and the latter with five million bacilli. The mesenteric glands of both animals illustrate the same point as the gland of Pig 26, *viz.*, the presence of capillaries in the midst of caseous areas containing bacilli.

LUNGS.

In relatively early cases, *e.g.*, Calf 288, killed 14 days after subcutaneous inoculation with virulent bacilli, small caseous foci containing bacilli are sometimes found directly bordering upon the lumen of dilated capillaries. It is often impossible to decide whether these foci have attacked the capillary wall by process of expansion, or whether they originated from bacilli escaping from the capillary wall; in either case they are in a position which allows the bacilli to return to the blood stream.

In the later stages of acute infection, where there is a general breaking down of the tissue and bacilli are freely distributed in large numbers, bacilli are sometimes found passing through the wall of a vessel and entering the lumen. I have found them, for example, in the lungs of Calf 238 (22 days after subcutaneous inoculation with 50 mg. of a virulent virus). And in the lungs of Calf 561 (33 days after subcutaneous inoculation with 50 mg. of a virulent human virus) one of my sections shows a rather earlier stage of the same process, *viz.*, a caseous tubercle, containing numerous bacilli, which has broken through a vessel wall and is bulging into the lumen, but has not yet discharged any of its contents into the vessel. In Monkey 2 (41 days after subcutaneous inoculation with bovine bacilli) a section of the lungs shows large groups of bacilli surrounding an artery; some of these have penetrated the muscular walls, and a few have arrived as far as the lumen; in other parts of the same section bacilli are seen passing from tubercles rich in bacilli into adjacent capillaries. In Chimpanzee 14 (63 days after feeding with 1 mg. of bovine bacilli) the lungs show progressive lesions in an earlier stage than in the preceding examples, and in one of these foci bacilli are seen passing through the walls of a blood-vessel and entering the lumen. In the lungs of Calf 319 (57 days after intravenous inoculation with human bacilli of low virulence for the bovine when inoculated subcutaneously) there are examples of bacilli passing from breaking down tubercles into the interlobular lymph spaces; and the same process is shown in the lungs of Calf 529 (13 days after intravenous inoculation with human bacilli which showed an increase of virulence after animal passage). These examples are quoted as illustrating the mode of progressive dissemination now under discussion; but it ought to be added that in acute infections of the lungs the bacilli find plenty of pabulum, as evinced by their abundant multiplication, in the tissues outside the blood-vessels; the blood channels contain a much less favourable nutrient medium, and the bacilli only grow into them accidentally and occasionally; and when the bacilli arrive in the lungs by way of the circulation they exhibit a decided tendency to grow out of this medium.

In the more chronic types of tubercles found in the lungs, capillary channels are frequently found in the outer zones of the lesions and sometimes in the interior, but I have not found any examples in histological sections of bacilli actually passing into them.

LIVERS.

The chances of bacilli escaping into the circulation depend very largely on the position of the tubercular foci, their opportunities being most favourable when the lesion borders upon a vein, particularly a hepatic vein. Lesions in these situations offering an escape for bacilli may be found (1) in early stages, where caseation is commencing and before there has been time for the formation of a demarcation zone, and (2) in later stages, where the lesions are progressive without evidence of conservative change. The liver of Pig 94 (61 days after subcutaneous inoculation with 1 mg. of bovine bacilli) shows several examples of bacilli escaping into veins.

The foci situated in the midst of parenchymatous cells and not bordering upon any vessels larger than capillaries may be divided roughly into (1) the more acute lesions, which are progressing without the formation of any demarcation zone, and (2) the less acute, which are surrounded by a peripheral zone of newly formed or adventitious cells. In (1) the blood capillaries rapidly disappear from the interior of the focus and the capillary obliteration generally keeps pace with the line of advancing necrosis; but bacilli are often found immediately beyond this line, and then, though the majority of them are detained by the endothelial or parenchymatous cells with which they come into contact, there is at least an opportunity for their escape into patent capillaries. The acutely infected livers found in calves, pigs, rabbits, and dogs afford examples of this condition. In (2), the tubercles which tend to become circumscribed, capillary obliteration also takes place concomitantly with the caseating process, but this obliteration is not always complete, and tubercles are sometimes found which, besides being vascular at their periphery, are not completely avascular in the centre. It is also to be noted that in lesions of the chronic type formation of new capillaries is often observed in the peripheral zone, and some of these vessels pass a short distance into the interior.

KIDNEYS.

A special feature of early tubercle formation in the kidney is that the lesions tend to spread irregularly along the intertubular tissue, rather than to extend by uniform expansion of a central focus. In this process the bacilli evidently follow the lines of least resistance. When caseation sets in it commences in these interstitial areas and gradually spreads to the tubules. Another consequence of the tendency of the disease to spread along tracks of interstitial infiltration is that blood-vessels are frequently surrounded by a tubercular infiltration and, as the disease progresses, may be isolated in the midst of caseous substance. This development of caseous tracts tending to surround blood-vessels occurs both in the cortex and in the medulla, and when the lesions are progressive and rich in bacilli is an obvious means of leading to dissemination by the blood-channels. The kidney of Calf 124 (died 71 days after subcutaneous inoculation with 9,000 bovine bacilli) may be quoted as an example. The largest lesions, situated in the cortex, are of irregular outline and exhibit advanced caseation with commencing calcification; the periphery of the lesions exhibit a zone of cellular infiltration. The smaller lesions are of the same type, and of irregular outline, but show less advanced necrosis, whilst the earliest consist merely of an instertitial infiltration. Tubercle bacilli are fairly numerous in the necrotic patches and sometimes form a ring surrounding blood-vessels which have escaped destruction in these areas. The older lesions contain more tubercle bacilli than the earlier.

MAMMARY GLANDS.

The cow's udder is a highly vascular organ and, as in the kidneys, tubercle bacilli penetrate very readily along the interstitial tissue between the lobules and alveoli. The consequence is that both capillaries and larger vessels frequently become surrounded with breaking down tissue containing numerous bacilli and must sooner or later give way, thereby affording the bacilli a chance of entering the blood-stream. The vascularity of the lesions, even in advanced infections, has been very noticeable in many of the udders which I have examined. As showing that this condition is not confined to rapidly progressive cases, special mention may be made of the udder of Cow 74 (299 days after intramammary inoculation) where I have found bacilli penetrating, and others actually within, dilated blood capillaries. The lesions in this udder are not of the acutely progressive type. There are large caseous tubercles, circular in outline and surrounded by a broad fibrous tissue zone; blood capillaries are conspicuous in the peripheral parts of these lesions, and the rest of the tissue is highly vascular, containing groups of normal mammary alveoli side by side with areas in which the alveoli have been partially replaced by tubercular infiltration. Bacilli are very abundant.

V.—By Transference of Foci from one Site to Another.

ENDOTHELIAL CELLS.

In considering the obstacles which arrest the dissemination of bacilli, I laid special stress on the cells forming the endothelium of lymphatic channels, of lymph sinuses in lymphatic glands, of blood capillaries, and of larger blood-vessels. Bacilli in the course of dissemination are constantly sticking to these cells. When the cells remain *in situ*, further changes arise, which I have already mentioned. But it very frequently happens that the cells become detached, and return into the circulation carrying the bacilli with them. As is seen by the study of early lesions, when a bacillus becomes attached to, or penetrates, an endothelial cell pathological alterations in the cell are usually produced very soon; and it seems probable that the modifications leading either (1) to the detention of the bacillus *in situ*, or (2) to the swelling up of the endothelial cell and its discharge into the circulation are effected quickly. I have found endothelial cells containing bacilli lying free within the lumen of blood-vessels in the calf's liver as early as the third day after subcutaneous inoculation. And in all stages of infection, early, intermediate, or terminal, where bacilli are demonstrable at all in the blood-stream, some of them, generally a considerable number of them, are found attached to, or within, detached endothelial cells. Amongst the commonest sites for the arrest of bacilli by endothelial cells are the lymph sinuses of lymphatic glands; in these situations bacilli-laden endothelial cells, detached, or in process of detachment, can frequently be found, particularly when drainage of bacilli into the gland is going on, or when lesions in the gland are breaking down and discharging bacilli into the sinuses. Probably many of these detached cells get carried on into the efferent lymph stream and thence into the blood-stream. This conveyance of bacilli by detached endothelial cells I have found taking place in all varieties of animals which I have examined for the purpose of studying dissemination, and it seems to me to play a very important part in the spread of the disease.

INTRAVASCULAR TUBERCLES.

Occasionally an intravascular tubercle is found attached to the lumen by a very narrow pedicle; it may then become detached as a whole, and be carried along by the blood-stream. But more usually these tubercles have a relatively broad area of implantation on the vessel wall and discharge their contents piecemeal, by caseous degeneration of their distal portion.

OTHER METHODS OF TRANSFERENCE.

For the sake of completeness mention ought to be made of some of the other important and obvious modes by which infected foci are conveyed from one site of the body to another. In the alveoli of the lungs clumps of desquamated epithelial cells, laden with bacilli, and usually containing an admixture of other cells, particularly leucocytes, are extremely common; these foci may travel along the air passages, spreading infection as they go, escape into the alimentary tract, and set up lesions in the intestine and elsewhere. Similarly, renal tubules and tubules of the mammary gland are frequently occupied by groups of cells laden with bacilli, which, as they are carried onwards, may continue the infective process.

D.—THE GENERAL CONDITIONS UNDER WHICH BACILLI CIRCULATE AND GROW.

I.—Circulating Bacilli.

The importance of the part played by the blood-stream in the dissemination of tubercle bacilli is clearly established, and numerous instances have been quoted in preceding paragraphs where this process is demonstrable in histological sections. But considering as a whole the total number of histological specimens examined, taken from various types of animals showing active disease, the presence of bacilli within sections of blood-vessels is an extremely rare event. They can only be found in a relatively few, specially selected specimens, and, when demonstrable, are often very scanty.

The blood-stream is an extremely unfavourable environment for the mammalian tubercle bacillus, and whenever bacilli find themselves in this situation they endeavour to get out of it, and generally disappear with remarkable rapidity. The discovery of a bacillus within the blood-stream generally means that active invasion of the circulation from some other source was going on at the time the specimen was obtained, or very shortly before; allowance must, however, be made for the fact that when bacilli become attached to endothelial cells, which afterwards escape from the vessel wall and are carried along in the circulation, there may be a longer delay before the bacillus is effectively eliminated from the blood-stream. Under ordinary circumstances, therefore, the passage of bacilli by the blood-stream is an intermittent event, the frequency of which is determined by, though not strictly synchronous with, the frequency with which lesions break down and allow their contents to be absorbed by the blood-stream. (Perhaps the absorption of bacilli through an intact mucous membrane ought to be added as a further contingency.)

Under exceptional conditions, circulating bacilli may be found in relatively large numbers. In calves fatally infected by subcutaneous inoculation with 50 mg. of highly virulent bacilli, this condition is occasionally found, *e.g.*, in a single section of the lung perhaps 40 or more intravascular bacilli may be seen. But out of a large number of specimens derived from terminal infections resulting from this method of inoculation, I have rarely found intravascular bacilli so numerous; in fact it is only in an occasional instance that any can be found, and in my earlier observations I failed to discover them. In the monkey, which is very much more susceptible than the calf both to subcutaneous infection and to feeding, intravascular bacilli are much more common in the terminal stages of the disease. In intravenous experiments, under conditions of virulence and amount of dosage which do not overwhelm the animal's resisting powers in the early stages, the bacilli soon escape from the blood-stream; but in young calves an overpowering effect may be produced by a large intravenous dose of bacilli relatively innocuous when introduced subcutaneously, and under these circumstances bacilli may be more readily found in the blood-stream, sometimes in fairly large numbers, though in other instances they have been scanty. Even in the most severe cases of calves infected by intravenous inoculation, intravascular bacilli have never been found in abundance. Monkeys are very highly susceptible to intravenous inoculation and often show large numbers of intravascular bacilli when fatally infected by this method. In rats, which tolerate the presence of enormous numbers of bacilli in their tissues without the production of tubercular lesions, bacilli are rarely found in the blood-stream, though the surrounding tissue be crowded with them. In cats, which sometimes exhibit, like the rat, a relative indifference to the presence of bacilli in their tissues, intravascular bacilli are more readily found, and may be fairly abundant. In the blood-vessels of dogs, at the terminal stage of infection when a rapid breaking down of caseous foci is going on, intravascular bacilli are moderately frequent.

II.—Relation of Circulating Bacilli to Cells.

FREE BACILLI.

Tubercle bacilli are not necessarily dependent on cells to carry them about in the circulation. They often circulate as free bacilli, without attachment either to cells or to any other material. The proportion of free to attached or intracellular bacilli varies very widely under different conditions. When bacilli are found at all, even if only in very small numbers, it rarely happens that they are all intracellular, either in early or in later periods after inoculation; and when the bacilli are rare, it generally is found that the proportion of bacilli attached to or within cells is relatively small. But in the exceptional cases where circulating bacilli are numerous, the free bacilli usually occur singly, whereas several may occur in each of a small group of cells; and hence the proportion of intracellular bacilli may be high.

CONVEYANCE BY CELLS.

Multinuclear Leucocytes.—It is only in very rare instances, and then in very small numbers, that I have found these cells conveying bacilli in the circulation. And in places showing very recent dissemination of bacilli from the circulation into the tissues the bacilli are not within multinuclear leucocytes, nor is there any appearance suggesting that they had been conveyed by cells of this type. On the other hand oxyphil leucocytes which have migrated into the tissues are often found very vigorously attacking and ingesting tubercle bacilli, particularly when the bacilli are numerous, or when they have set up early tissue changes. Under these circumstances, of which the local lesions removed from calves afford excellent examples, and the early disseminated lesions in pigs likewise, oxyphil leucocytes containing bacilli are extremely numerous; and when the supply of bacilli is plentiful many of the leucocytes are absolutely stuffed with them. But oxyphil leucocytes which have ingested tubercle bacilli suffer profound pathological changes, as evinced by the rapid loss of their protoplasmic staining reaction and the rapid disintegration of their protoplasm; when not actually dead, they are at least in a very helpless condition, and are probably quite unequal to the task of returning to the circulation with a tubercle bacillus inside them. This is the explanation of the very striking difference between the frequent presence of bacilli within these cells in the tissues, amongst the exudates of lung alveoli, mammary alveoli, and elsewhere, and the extreme rarity of bacilli within the bodies of multinuclear leucocytes circulating in the blood-stream. My observations lend no support to the theory, sometimes put forward, that oxyphil leucocytes play an important part in the conveyance of bacilli from one region of the body to another.

Detached Endothelial Cells.—These cells are by far the commonest and most important vehicle for conveying cells from one part of the body to another. In the blood-stream the cells containing or adherent to bacilli are nearly all of this nature.

Other Cells or Cellular Débris.—A tubercle bacillus may stick to anything, to a red corpuscle, or a small lymphocyte, or to any other cells, fragments of cells, or non-cellular débris, which may happen to be in the circulation. But conveyance by any of these methods is only accidental and occasional. There is no vehicle of transmission which is employed so regularly and so characteristically as the endothelial cell.

EXAMPLES OF FREE AND ATTACHED BACILLI.

In a section of a monkey's lung (Monkey 2) 140 circulating bacilli were counted; 137 of these were attached to large mononuclear cells, and the remaining 3 were free. In the tissues of 5 other monkeys, 40 circulating bacilli were counted; 26 of these were attached to large mononuclear cells, one was within a multinuclear cell, and 13 were free.

In the tissues of 6 cats, 245 circulating bacilli were counted; 95 of these were attached to large mononuclear cells, 2 were within multinuclear cells, 28 were attached to other cellular material, and 120 were free.

In the tissues of 2 dogs, after intraperitoneal inoculation, 77 circulating bacilli were counted; 69 of these were attached to large mononuclear cells, 2 were within multinuclear cells, and 6 were free.

In 27 tissues obtained from 19 bovines, 300 circulating bacilli were counted; 58 of these were within large mononuclear cells, 6 were within multinuclear cells, 13 were attached to other cellular material, and 223 were free. The percentage of free cells was therefore 74. It is interesting to compare this result with the condition found in a film of tuberculous milk, obtained from Cow 74. The film was found to contain 4,006 bacilli; 1,006 of these were attached to cells or cellular material, and the remaining 3,000 were free. The percentage of free bacilli was therefore 75.

III.—The Growth of Bacilli in the Body.

Histological observations clearly show that the blood-stream is a highly unfavourable medium for the growth of the bacillus; in fact the bacilli which find their way into this medium do not seem able to survive in it for very long, and I have never met with an example indicating that they are multiplying in it. In some of my bacteriological experiments I have attempted to cultivate the bacilli on pure undiluted serum in the liquid state, but only obtained a scanty yield; these observations have not been very numerous, but they may be associated with the strong histological evidence that the bacillus seeks to avoid the blood-stream. On the other hand the tubercle bacillus grows well in ordinary cow's milk, sterilized but otherwise unaltered, when incubated in a test-tube at 37°; and it retains its vitality in this medium for a very long period (over a year) without subculture. It is interesting to associate this fact with the abundance of bacilli often observed within the tubules, in the ducts, and in the milk sinuses of infected udders. It also supports the evidence that bacilli may lodge and multiply for a long period within the pockets of a milk sinus, although at the post-mortem no evidence of tuberculosis can be found either macroscopically or microscopically. The udders of Cows 73 and the spontaneously infected animals, Cows B and C, may be quoted as examples.

With regard to the capacities of individual cells for affording nutriment to the bacillus marked differences are found. The bacilli are clearly unable to thrive within the multinuclear leucocyte, the invariable result of bacilli finding their way into these cells being rapid disintegration, either of the cell, or of the bacillus, or of both. In endothelial and connective tissue cells, growth of the bacilli is possible without cell disruption, and the protoplasm of cells belonging to these types often contains bacilli in considerable numbers. And occasionally intracellular bacilli are very abundant but have produced little evidence of tissue destruction. This phenomenon is sometimes ascribed to the intensely phagocytic action of the cells, rather a misleading term, since the bacilli are thriving upon the cell much more vigorously than the cell is upon them. With regard to epithelial cells and other types, all that need be said here is that the bacilli may in most cases obtain nutriment from them and grow in their protoplasm, but not without establishing degenerative changes in the cell substance. Within cell fusions and within definite giant cells bacilli may retain their vitality for a long time, and evidence of multiplication in these sites is very often found. But tissue which is so far disintegrated that cell details can no longer be made out is generally much the most favourable site for the proliferation of the bacilli.

II.—THE REACTION OF THE ANIMAL ORGANISM.

A.—THE RELATIONS OF OXYPHIL* LEUCOCYTES TO MAMMALIAN TUBERCLE BACILLI.

I. IN BOVINES.

(1.) At the site of Subcutaneous Inoculation.

AFTER TWENTY-FOUR HOURS.

Calf 438.—Dose, 44 mg. of bovine bacilli. The mass of inoculated bacilli is invaded by enormous numbers of multinuclear leucocytes, which are actively phagocytic. Many of the leucocytes are crammed with bacilli, though there are also many leucocytes which are present in the neighbourhood of bacilli but do not contain any. The leucocytes are evidently streaming in the direction of the bacilli; there is no indication that leucocytes are conveying bacilli back into the blood-stream.

AFTER THREE DAYS.

Calf 284.—Dose, 50 mg. of bovine bacilli. As in the previous case, the mass of bacilli is permeated by enormous numbers of leucocytes, which are actively phagocytic. At the periphery of the foci showing dense groups of bacilli, relatively few of the leucocytes contain bacilli; and, passing outwards into the areas of commencing infiltration with bacilli, it is observed that the leucocytes, though numerous, have rarely ingested any. In the lymph channels leucocytes containing bacilli have occasionally been found. The leucocytes are almost all of the finely granular or non-granular type; very few coarsely granulars are present; none of these latter contain bacilli.

Calf 873.—Dose, 50 mg. of human bacilli of low virulence for the bovine. Identical with the condition of Calf 284.

AFTER EIGHT DAYS.

Calf 292.—Dose, 50 mg. of bovine bacilli. Actively phagocytic leucocytes are still abundantly present amongst the masses of bacilli. Many of the leucocytes are broken into fragments. In areas where bacilli are rarer, and occur either singly or in only small groups, leucocytes, though plentiful, very rarely contain bacilli. A few coarsely granulars are present, but do not contain bacilli. Amongst the phagocytic, non-granular or only finely granular leucocytes, forms with a spherical nucleus are common.

Calf 881.—Dose, 50 mg. of human bacilli of low virulence for the bovine. Phagocytic activity of the leucocytes is well shown, as in Calf 292. But the granules of the leucocytes (the ordinary phagocytic forms) are coarser than in Calf 292 or Calf 873 (the three days' specimen). And, compared with the three days' specimens, leucocytes are less numerous, whilst the proportion of other cells is greater.

AFTER FOURTEEN DAYS.

Calf 288.—Dose, 50 mg. of bovine bacilli. Both the multinuclear and the mononuclear forms of oxyphil leucocytes are abundant at the periphery of the lesions; in the older foci the majority of them are broken down. Many of the leucocytes contain bacilli, and at the outskirts of the lesion there is more evidence of vigorous phagocytic action than in earlier specimens. But no oxyphils containing bacilli have been found within intact blood vessels. Leucocytes with a distinct granulation are rarely found.

Calf 869.—Dose, 50 mg. of human bacilli of low virulence for the bovine. Tubercle bacilli are relatively scanty in the walls of the lesion; (the central portions had been reduced to a liquid state). The bacilli generally occur within fixed tissue cells; none have been found within leucocytes. Oxyphil leucocytes are rarer than in the corresponding eight days' specimen (Calf 881); frequently, but not invariably, they are markedly granular.

AT LATER PERIODS.

Calf 769.—Died, from intercurrent disease, 17 days after inoculation with 50 mg. of human bacilli of low virulence for the bovine. Bacilli are numerous and are freely disseminated in the lesion; leucocytes, all of the finely granular or non-granular type, are abundant in all parts, and often contain tubercle bacilli. There are numerous blood-vessels in the lesion, but the leucocytes present in these situations do not contain bacilli.

Calf 767.—Killed 25 days after inoculation with 50 mg. of human bacilli of low virulence for the bovine. The lesion consists of fibro-caseous material and shows numerous bacilli in some of the caseous areas. Leucocytes, non-granular or only faintly granular and often broken into fragments, are numerous in the lesions; some of them, but only a relatively small number, contain bacilli.

Calf 185.—Killed 40 days after inoculation with ·75 cc. of bacilli derived from a human virus of low virulence for the bovine. The lesion contains large necrotic foci which are partially circumscribed. Bacilli are found both in the centre and at the periphery of the lesions, and are moderately numerous, particularly in the necrotic foci. At the periphery of the lesions leucocytes are fairly numerous and generally show a distinct but not a coarse granulation; in the central areas broken down leucocytes and also leucocytes which give a good protoplasmic stain are plentiful. Occasionally, but very rarely, one or two bacilli have been found within leucocytes.

Calf 105.—Killed, when moribund, 47 days after inoculation with an emulsion estimated to contain 48,000 bacilli; the bacilli were derived from a human virus of high virulence for the bovine. The lesion is of the caseating, slowly progressive type. Bacilli are scanty. Leucocytes are fairly plentiful, and in one of them a bacillus has been found. The leucocytic infiltration appears to be caused by the breaking down tissue rather than by the actual presence of bacilli.

Calf 871.—Killed 91 days after inoculation with 50 mg. of a human culture of low virulence for the bovine. The wall of the local lesion (the central portion being liquid) consists of dense fibro-caseous material with some calcareous deposits. Bacilli are scanty; none have been found within leucocytes. In the caseating areas leucocytes and broken-down leucocytes are plentiful; amongst the cells of the peripheral zone they are also numerous and are generally definitely granular.

Calf 510.—Killed 96 days after inoculation with 50 mg. of bacilli, isolated from an antelope, possessing low virulence for the bovine. The local lesion is separated from the underlying muscle by a dense fibrous zone; internal to this is a layer of loosely arranged cells, becoming caseous as we pass inwards. In this cellular layer bacilli are numerous and are generally contained within cells, but not within leucocytes. Leucocytes, some faintly and some markedly granular, are freely distributed in this area, but show no signs either of converging upon the bacilli or of ingesting them.

Calf 267.—Killed 111 days after inoculation with 3 gms. of bacilli, killed by heating, derived from a human virus of low virulence for the bovine. The lesion shows patches of slight caseation, amongst which bacilli are occasionally to be found. Throughout the specimen, leucocytes, with a well-marked granulation, are fairly numerous; they show no relationship to the bacilli.

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* Under the term "oxyphil" I include the cells with less strongly marked oxyphil characters which are termed by some writers "neutrophil."

(2.) In the Lungs.

EARLY LESIONS.

Calf 284.—Killed three days after subcutaneous inoculation with 50 mg. of bovine bacilli. The only lesions noted are small patches showing slight thickening and congestion of the alveolar walls. Bacilli are very rare. There is a slight excess of multinuclear leucocytes in these situations, but no bacilli have been found within leucocytes.

Calf 292.—Killed eight days after subcutaneous inoculation with 50 mg. of bovine bacilli. In several of the alveoli bacilli are present, and have produced lesions which vary in extent from a slight thickening of the alveolar wall to a tubercle about twice the diameter of an ordinary alveolus. Leucocytes are in excess of the normal but not very abundant. They occur in all the foci where bacilli are present, being scanty in the smaller and more numerous in the larger.

Calf 288.—Killed 14 days after subcutaneous inoculation with 50 mg. of bovine bacilli. Early lesions are very numerous. The largest are definite tubercles, about four times the size of an ordinary alveolus; the smallest are merely slight thickenings of the alveolar walls. Bacilli are present in all the lesions, and the largest may contain from 50 to 100. Leucocytes are present, but not very numerous, in these situations; none of the leucocytes have been found containing bacilli. Some of the leucocytes are broken down, but there is no evidence that a large destruction of these cells has taken place.

Calf 869.—Killed 14 days after subcutaneous inoculation with 50 mg. of human bacilli of low virulence for the bovine. A few small, early lesions are present. In two of them, each about the size of an alveolus, and each containing four or five bacilli, there are many leucocytes and broken-down leucocytes; though very few have been found actually containing bacilli these cells may have been partly responsible for the rarity of bacilli.

LATER LESIONS—ACUTE.

Calf 436.—Died 16 days after subcutaneous inoculation with 50 mg. of an equine virus of high virulence for the bovine. The cause of death was not determined. The lungs contain numerous early tubercles containing moderately large numbers of bacilli. In the tissue generally there are more leucocytes than normal, and in the tubercles which are commencing to caseate there is a moderate though not copious infiltration of these cells, which appear to be attracted towards the bacilli.

Calf 244.—Died 19 days after intramuscular inoculation with 50 mg. of a bovine virus. The lesions in the lungs are of the usual acute type found in fatal infections, though not so advanced as when the animals have survived rather longer. The tubercles contain large numbers of bacilli and are rapidly breaking down. In the tissue external to the tubercles finely granular oxyphil leucocytes are very numerous and take a good eosin stain; the nuclei of some of them appear to be dividing amitotically. Within the lesions these cells, many of them broken down, are also very numerous, but their protoplasm stains more faintly and many of them fail to take any protoplasmic stain. The general appearance indicates that leucocytes are being attracted to the lesions in large numbers, but rapidly break down when they come into contact with the bacilli. Many leucocytes contain bacilli.

Calf 250.—Died 30 days after subcutaneous inoculation with 50 mg. of bovine culture. The lungs show large patches of diffuse necrotic change, the intermediate areas being highly congested and containing a considerable amount of exudate. Bacilli are very numerous in the lesions. Leucocytes are numerous throughout the tissue; the lesions are abundantly invaded with these cells, but in these situations the majority of them have broken down. A fair number, however, of the leucocytes which retain an oxyphil stain contain bacilli.

Calf 272.—Killed, when moribund, 46 days after subcutaneous inoculation with 50 mg. of bovine bacilli. The lungs show the usual condition of diffuse necrotic change, with numerous bacilli. In the lesions there are many signs of broken-down leucocytes, but at their periphery and in the surrounding tissue leucocytes giving a good protoplasmic stain are not very numerous. The general appearance indicates that there has been no recent invasion of these cells in large numbers. In the lungs obtained from some of the other experiments which terminated at about this period after inoculation there seems to have been a lull in the process of leucocytic invasion.

Calf 779.—Died 63 days after subcutaneous inoculation with 10 mg. of a strain of human bacilli possessing high virulence for the bovine. The tissue is typical of acute infection. At the periphery of the lesions bacilli are very numerous and are surrounded by large numbers of leucocytes, many of which are disintegrated. Throughout the specimen leucocytes are numerous and appear to be attracted towards the bacilli, several leucocytes giving a good oxyphil stain actually contain bacilli.

Calf 440.—Died 72 days after subcutaneous inoculation with 50 mg. of an equine virus of high virulence for the bovine. The tissue is typical of the terminal stage of acute infection. Masses of leucocytes and their remains are present at the margin of the necrotic areas. Leucocytes are also numerous in the tissue intermediate between the lesions. The leucocytes are vigorously attacking and ingesting the bacilli, which are very abundant, and sometimes occur within cells retaining an oxyphil stain.

Calf 498.—Died 89 days after subcutaneous inoculation with an equine virus which in some experiments has proved of only slight virulence for the bovine. The lungs are acutely infected. Both bacilli and leucocytes are very numerous. The relation of the leucocytes to the bacilli is the same as in the previous animal, Calf 440. Occasionally leucocytes retaining an oxyphil stain contain bacilli.

Calf 448.—Killed 104 days after subcutaneous inoculation with 50 mg. of porcine bacilli possessing relatively high virulence for the bovine. The lungs exhibit severe, progressive infection, but with less necrosis than is usually found in very acute cases. Both bacilli and leucocytes are abundant. The leucocytes appear to be definitely attacking the bacilli; where the bacilli form large groups, dense masses of these cells are present, many of them broken down. Leucocytes giving an oxyphil stain not unfrequently contain bacilli.

Cow 74.—Died 299 days after an intramammary inoculation with an emulsion of bovine bacilli, probably scanty in number. The lungs are acutely infected, exhibit large, ramifying caseous areas, and contain very large numbers of bacilli. Leucocytes are very abundant and are evidently attacking the bacilli. Cells retaining an oxyphil stain sometimes contain them.

LATER LESIONS—OF INTERMEDIATE SEVERITY.

Calf 426.—Died 29 days after subcutaneous inoculation with 200 mg. of culture of a porcine virus possessing a relatively low virulence for the bovine. The lesions are much less extensive than in highly acute infections, but the alveolar walls show a general thickening, and many small tubercles, in an early stage of caseation, are present. Throughout the tissue there is a considerable degree of leucocytosis; within the tubercles many of the leucocytes are broken down, but fresh leucocytes appear to be passing into the lesions from the periphery. Bacilli are moderately numerous and sometimes occur within thickened alveolar walls apart from definite tubercles. When present in the former situations, the bacilli are not usually associated with leucocytes; but where definite tissue disintegration has been set up, bacilli and leucocytes are found together. With a few exceptions which are coarsely granular, the leucocytes are of the finely granular type, but their granulation is more distinct than is the case in highly acute infections. Bacilli are found within leucocytes retaining an oxyphil stain.

Calf 52.—Killed 32 days after subcutaneous inoculation with an emulsion of bacilli derived from a bovine virus. Throughout the specimen are small areas of consolidated tissue, irregular in outline and without demarcation by fibrous zones; many of the lesions are centrally caseous. The lesions are evidently progressive, but not of a very acute type; bacilli are not very numerous and are confined to the lesions, chiefly to the necrotic areas. Oxyphil leucocytes, both multinuclear and mononuclear are present in large numbers, both in the consolidated areas and in the rest of the tissue, their numbers being greatest at the periphery of the lesions, and not in immediate contact with bacilli. The leucocytes are finely granular or non-granular.

Cow 63.—Died 45 days after subcutaneous inoculation with an emulsion of bacilli, estimated at 160 million, derived from a virus isolated from human sputum. The lesions are slightly caseous, not sharply circumscribed, and surrounded by a broad cellular, but not by a fibrous, zone. There is no indication that they are progressing rapidly. Bacilli are very scanty. The lesions are infiltrated with large numbers of leucocytes.

Calf 281.—Killed, when ill, 54 days after subcutaneous inoculation with an emulsion of bacilli, estimated at 11 million, derived from a human virus which became of high virulence for the bovine after repeated passage. The tissue contains numerous tubercles. Some of the older and larger are partially marked off from the surrounding tissue by a layer of fibroblasts; but in the younger tubercles, which are more numerous and quite irregular in outline, this demarcating layer is absent. Leucocytes are numerous within the lesions (both the older and the more recent), at their periphery, and in the surrounding tissue. The majority of the leucocytes are granular, and many of them possess a distinctly coarse granulation. Bacilli are not very numerous, but are rather more frequent in the older lesions than the earlier. There is no marked association between the leucocytes and the bacilli. The leucocytes are invading the affected tissue as a whole and not specially converging upon the bacilli.

Calf 365.—Killed 61 days after subcutaneous inoculation with an emulsion of human lung, estimated to contain about 100 million bacilli. Small tubercles occur abundantly throughout the tissue examined; they contain many giant cells, and some of the larger are commencing to caseate. There is also a catarrhal thickening of the alveolar walls. The older tubercles are generally discrete, but the earlier ones are often continuous with the catarrhal patches. Bacilli are rare. Leucocytes are abundant both in the catarrhal areas and within the tubercles, particularly in the latter situations, where they often form large masses. Both non-granular, finely granular, and coarsely granular leucocytes are found.

Calf 312.—Killed 90 days after subcutaneous inoculation with 50 mg. of a porcine virus of relatively low virulence for the bovine. The lungs contain irregular, ramifying, caseous tubercles with a broad cellular margin; smaller lesions, infiltrated with cells but not caseous, are also present. Bacilli are very numerous, particularly in the caseous areas. Leucocytes, most of them finely granular, are present in all the lesions, generally in large numbers. Round the margins of the caseous areas they are particularly abundant; passing inwards towards the caseous material most of them are fragmentary; the disintegration of the leucocytes appears to be definitely associated with the presence of bacilli. Many of the leucocytes, including those which retain an oxyphil stain, contain bacilli.

Calf 340.—Killed 92 days after subcutaneous inoculation with 10 mg. of a bovine virus. The portion of lung examined is almost completely solidified and is undergoing fibroid change. It contains several caseous patches; some of these are calcareous in the centre and are surrounded by a broad fibrous zone; but in many situations caseation is only just commencing, and there is no demarcation into tubercles. Bacilli are scanty but are not confined to the caseating areas. Leucocytes are very numerous throughout the tissue and penetrate into the caseous material. The granulation of the leucocytes is well marked, and in many of them is distinctly coarse.

Calf 905.—Killed 90 days after subcutaneous inoculation with 50 mg. of human bacilli of relatively low virulence for the bovine. The tissue shows a patchy consolidation with many caseous areas, some of them containing slight calcareous deposits. There is an irregular formation of fibrous tissue, but the lesions are not definitely circumscribed. Bacilli are present in all the lesions, and in some of the foci are numerous. Leucocytes are much less numerous than lymphocytes, but occur in all parts of the tissue; some of them are coarsely granular. The leucocytes are distributed irregularly and do not form special aggregations in the neighbourhood of the bacilli.

Calf 474.—Killed 92 days after subcutaneous inoculation with 50 mg. of bovine bacilli. The tissue contains consolidated areas of the slowly progressive type, centrally caseous, but abundantly infiltrated with cells, amongst which leucocytes are less numerous than lymphocytes. Bacilli are not numerous and are not associated with special aggregations of leucocytes.

LATER LESIONS—CHRONIC.

Calf 767.—Killed 25 days after subcutaneous inoculation with 50 mg. of human bacilli of low virulence for the bovine. In specimens selected from different parts of the lungs the greater portion of the tissue is normal, but a few small tubercles are present. No bacilli have been found in them. The smallest of the tubercles show no sign of caseation but are completely overrun with cells, amongst which leucocytes and lymphocytes are present in almost equal numbers. In the rather larger foci there are slight traces of caseation; these lesions are swarming with leucocytes, whereas lymphocytes are scarce. The leucocytes, hardly any of which show nuclear disintegration, are mostly of the finely granular type, but a few possess coarser granules and some of these cells have shed their granules.

Bull 88.—Killed 45 days after subcutaneous inoculation with 50 mg. of a bovine virus. Sections of the lungs show many large, partially caseous tubercles which contain very few bacilli, have broad cellular margins containing many fibroblasts, and are in all respects retrogressive in character. The leucocytes show no relationship to the bacilli present but are very numerous in the cellular portions of the tubercles; most of them are granular; the granules vary in size, some being small, others rather larger, and others definitely coarse; no sharp distinction can be drawn between these varieties.

Calf 240.—Killed 54 days after subcutaneous inoculation with 50 mg. of bovine bacilli. The specimen of lung contains five small, retrogressive tubercles in which bacilli are very scanty. The tubercles contain a copious infiltration of cells, amongst which oxyphil leucocytes occur, though these cells are much less numerous than the lymphocytes.

Calf 165.—Killed 57 days after subcutaneous inoculation with an emulsion of bacilli, estimated at about 4½ million, derived from a human virus of low virulence for the bovine. Specimens from two different parts of the lungs show several small, retrogressive tubercles, containing very few bacilli. As in the previous specimen, Calf 240, the lesions are copiously overrun with cells, but the number of leucocytes present is small in proportion to that of the lymphocytes.

Calf 525.—Killed 90 days after subcutaneous inoculation with 50 mg. of human bacilli belonging to a virus usually of high virulence for the bovine. The animal in this instance was six months old, *i.e.* much older than the calves usually employed for testing virulence. The specimen of lung contains five tubercles, each about half the diameter of the microscopic field (low power). The tubercles, in which a few bacilli are present, contain a small caseous patch in the centre; the remainder of the lesions is occupied by a mass of cells which are almost entirely small lymphocytes, but amongst these cells a few oxyphil leucocytes occur; and in some of the caseous centres a few leucocytes, retaining a good eosinophil stain, have survived.

Calf 667.—Killed 90 days after subcutaneous inoculation with 50 mg. of a human virus usually of high virulence for the bovine. Calf 667 was a young animal, no older than those usually employed for the virulence tests. The lesions in the lungs are minute obviously retrogressive, and, as regards the relationship of leucocytes to lymphocytes and to bacilli, identical with the previous animal, Calf 525.

Calf 360.—Killed 91 days after subcutaneous inoculation with 10 mg. of a bovine virus. The lesions in the lungs are minute and scanty and, as regards the relationship of leucocytes to the tubercles, are identical with the two preceding cases, Calves 525 and 667. Bacilli are rare and are only found amongst the caseous centres of the tubercles, not associated with leucocytes.

Heifer 30.—Killed 115 days after subcutaneous inoculation with an emulsion of tissue containing bovine bacilli, probably scanty in number. The lungs contain typical chronic, caseous tubercles, surronnded by a broad fibrous zone and containing very few bacilli. In the peripheral portions of the nodules oxyphil leucocytes are very abundant and are markedly granular; the nuclei of these cells present many varieties, the spherical, the multilobular, the deeply stained, and the lightly stained all being found.

AFTER INTRAVENOUS INOCULATION.

Calf 529.—Died 13 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 1,000 million, derived from a virus isolated from human sputum. The tissue is in a condition of progressive consolidation; in one part of the lung the consolidation is nearly complete; in a specimen from another part the general condition is the same, but the process is less advanced and the air-containing spaces are more numerous. The whole tissue is densely infiltrated with leucocytes, many of them broken down. The granules of many of the leucocytes are very distinct and not particularly coarse, although the cell nuclei are often larger and paler than in the typical finely granular multinuclear. Probably these cells represent a degenerating stage of the ordinary phagocytic leucocyte. The tissue is swarming with bacilli, which are being vigorously attacked by the leucocytes. Some of the cells retaining an oxyphil stain contain bacilli.

Calf 831.—Died 17 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 1,000 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. Consolidation is less advanced than in the preceding specimen, but the lesions are of the same type; the leucocytic infiltration is less copious, and the bacilli, though very numerous, are less abundant than in the preceding specimen, and have caused less tissue destruction.

Calf 89.—Killed, when very ill, 23 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 300,000, derived from a virus isolated from human sputum. The lungs are in a condition of progressive miliary tuberculosis. The lesions are commencing to caseate and contain numerous bacilli. Multinuclear leucocytes are abundant; in the middle of the lesions, where bacilli are most numerous, most of the leucocytes are broken down and have lost their granulation; but in other situations, a granulation can be made out with the oil-immersion lens; the granules show a tendency to escape from the cell body.

Calf 749.—Killed, when very ill, 24 days after intravenous inoculation with 46 mg. of a culture derived from a human virus of low virulence for the bovine when inoculated subcutaneously. The tissue shows generalised consolidation, without any caseous change or other histological indications typical of tuberculosis. Bacilli are scattered all over the specimen in fairly large numbers; there is also a general and very abundant distribution of leucocytes, but these cells do not seem specially attracted to the bacilli, which are often contained within, or adherent to, cells of other types.

Heifer 8.—Killed, when very ill, 25 days after intravenous inoculation with a tissue emulsion containing bovine bacilli. The lungs contain large patches of consolidation which are of the inflammatory type and do not exhibit tubercle formation. Tubercle bacilli are very numerous and are generally distributed. There is also a general dissemination of leucocytes in large numbers; the leucocytes not infrequently contain bacilli, and have lost their granulation where they form groups aggregated round the bacilli.

Calf 305.—Killed, when moribund, 26 days after intravenous inoculation with an emulsion of bacilli, estimated at 730 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. There is a generalised consolidation, of the same type as in Heifer 8, but rather less advanced. There is also a similar distribution of bacilli and leucocytes, but the bacilli are less numerous. The leucocytes are particularly numerous where the bacilli have caused early necrotic change in the tissue. Most of the leucocytes are granular or slightly granular. A few bacilli have been found within leucocytes giving an oxyphil stain.

Calf 399.—Killed, when very ill, 34 days after intravenous inoculation with an emulsion of bacilli, estimated at about 700 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. There is the same generalised process of consolidation without tubercle formation as in the last animal, but the process is rather more advanced. Bacilli are very abundant, and there is a generalised distribution of leucocytes, without any marked indication that the leucocytes are converging upon the bacilli.

Calf 101.—Killed, when moribund, 41 days after intravenous inoculation with an emulsion of bacilli, estimated at 48,000, derived from a human virus of high virulence for the bovine. Small tubercles are disseminated throughout the lung and are tending to become confluent. In many of them, bacilli are numerous. In the interior of the lesions there are many signs of broken-down leucocytes, but there is no evidence of recent invasion.

Calf 607.—Killed 48 days after intravenous inoculation with 90 mg. of culture of human bacilli derived from sputum. There are no tubercles, and only very few bacilli have been found. In the alveolar walls, many of which are thickened, leucocytes are in excess of the normal, but not very numerous.

Calf 319.—Killed, when moribund, 57 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at over 100 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. The tissue is densely packed with small, miliary tubercles. The tubercles contain very large numbers of bacilli and are crowded with leucocytes, many of which are broken down; but a few show a well marked granulation. Many of the leucocytes are actively phagocytic.

Calf 685.—Killed 64 days after intravenous inoculation with 10 mg. of a virus derived from human sputum. The tissue shows some general thickening of the alveolar walls and contains a few tubercles. These are of a retrogressive character, being centrally caseous and circumscribed by fibroblasts and an infiltration of wandering cells. Bacilli are scanty and are confined to the central parts of the tubercles, where they are surrounded by leucocytes and nuclear débris. In the peripheral cellular area of the tubercles leucocytes are scanty and lymphocytes are greatly in the majority.

Calf 361.—Killed 76 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 41½ million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. The lung is crowded with typical tubercles, which are caseous, contain giant cells, and are surrounded by a broad fibrous zone. Bacilli occur in many of the tubercles, but are scanty. Leucocytes are present at the margins of the caseous tubercles, and also in the centre of the less necrotic lesions, but are not numerous in proportion to other cells and are not found in association with bacilli.

Heifer 26.—Killed 115 days after intravenous inoculation with an emulsion of tissue containing bovine bacilli, probably scanty in number. The lungs contain circumscribed, isolated tubercles, surrounded by a very broad fibrous zone, and obviously chronic in character. A few bacilli are present in the caseous centres, but none have been found elsewhere. The cellular portions of the caseous tubercles are packed with an enormous number of eosinophil cells; the granules of these vary in size, the majority of them being very coarse.

(3.) In the Liver.

EARLY LESIONS.

Calves 284 and 873.—Killed three days after subcutaneous inoculation, each with 50 mg. of bacilli. Bacilli are rare and are not found associated with leucocytes, though small collections of these cells are present in several parts of the specimens.

Calf 292.—Killed eight days after subcutaneous inoculation with 50 mg. of bovine bacilli. Bacilli are rare. A few leucocytes are generally found near to them, but no bacilli have been found actually within leucocytes. In a few places the bacilli have set up early lesions, characterised by the obliteration of the parenchymatous cells, an increase of the endothelial element, and an infiltration of wandering cells; amongst these wandering cells leucocytes are present, but not in large numbers. And in the tissue generally, apart from lesions or bacilli, the leucocytes are in excess of the normal. The general appearance indicates a slight but not a vigorous determination of leucocytes towards the bacilli or towards the places where the bacilli have induced tissue changes.

Calf 288.—Killed 14 days after subcutaneous inoculation with 50 mg. of bovine bacilli. The lesions are more numerous and rather more advanced than those found in Calf 292; there is rather more disintegration of the parenchymatous cells and a more abundant infiltration of these areas with leucocytes. In some of the lesions the invasion of leucocytes appears to have been quite recent, as these cells are fairly numerous and all possess a well stained, finely granular protoplasm; in other lesions there are broken-down leucocytes but only a few with intact protoplasm; and in others, again, leucocytes are scanty and there is no evidence of the nuclear remains of these cells. Bacilli can be found in many of the lesions but only in small numbers. The attraction of the leucocytes towards the sites where bacilli are present is more pronounced than in the eight days' specimen, but none of the bacilli have been found actually within leucocytes.

Calf 869.—Killed 14 days after subcutaneous inoculation with 50 mg. of human bacilli possessing low virulence for the bovine. Lesions are much less numerous in this liver than in that of the 14 days' animal inoculated with a more virulent virus. In one specimen there is a small tubercle which is centrally caseous and is infiltrated with a large number of leucocytes; five bacilli can be seen in a section of this lesion, but none of them are in immediate contact with leucocytes. The lesion is in a rather more advanced stage of caseation than those found in Calf 288, and it is noteworthy that it has been infiltrated by a much larger number of leucocytes.

LATER LESIONS—ACUTE.

Calf 436.—Died 16 days after subcutaneous inoculation with 50 mg. of an equine virus found, in other experiments also, to be of high virulence for the bovine. There are many small lesions, centrally caseous and not circumscribed. They all show evidence of leucocytic infiltration; a great many of the leucocytes are broken down. Bacilli are present in all the lesions but are not very numerous; they have evidently been unable to multiply freely. Cells giving an oxyphil stain are not numerous except in the lesions; occasionally, but rarely, they contain bacilli.

Calf 244.—Died 19 days after intramuscular inoculation with 50 mg. of a bovine virus. The lesions are of the same type as in the preceding case but rather more extensive, and contain more bacilli. There is well marked leucocytic infiltration into the lesions. Many of the leucocytes are broken down, but there are also many, both in the interior and at the margins of the lesions, which show a good protoplasmic stain; the process of leucocytic invasion therefore appears to be still actively going on. Bacilli are not demonstrable in any of the cells giving a good oxyphil stain.

Calf 250.—Died 30 days after subcutaneous inoculation with 50 mg. of bovine culture. The lesions are larger than in Calf 244, contain bacilli in greatly increased numbers, and are obviously expanding. In addition to a large number of broken down leucocytes within the lesions, leucocytes with well stained protoplasm are also demonstrable in these situations, but are less numerous than in Calf 244; the activity of the leucocytic infiltration appears to be falling off, and outside the lesions these cells are scanty. Within the lesions, many of the cells giving a good protoplasmic stain contain bacilli.

Calf 352.—Died 40 days after subcutaneous inoculation with 10 mg. of a bovine virus. The lesions are of the ordinary progressive type. At their margins leucocytes are moderately numerous, the majority being broken down. Bacilli are less numerous than in Calf 250.

Calf 360.—Killed 91 days after subcutaneous inoculation with 10 mg. of a bovine virus. The lesions are of the same type as in the last animal, but bacilli are much more abundant, and at the periphery of the lesions numerous leucocytes are found; a considerable number of these cells are ingesting bacilli. Apparently the multiplication of bacilli has been coincident with an increased activity on the part of the leucocytes.

Calf 965.—Killed, when moribund, 60 days after subcutaneous inoculation with a human virus of high virulence for the bovine. Caseous, progressive lesions are present, but, compared with other lesions of the acute type, bacilli are not very numerous. Leucocytes, the great majority of which fail to take a protoplasmic stain, are numerous at the periphery of the lesions.

Calf 693.—Died 63 days after subcutaneous inoculation with 50 mg. of human bacilli possessing high virulence for the bovine. Caseous, progressive lesions of the ordinary acute type are present. At their periphery bacilli are numerous and in this situation leucocytes are to be found; though most of them are broken down, a smaller number take an oxyphil stain. The general appearance indicates only a slight degree of activity on the part of the leucocytes.

LATER LESIONS—INTERMEDIATE.

Calf 426.—Died 29 days after subcutaneous inoculation with 200 mg. of a porcine virus which, in the majority of experiments was found to be of relatively low virulence for the bovine. The liver contains numerous minute, slightly caseous foci, in which bacilli are scanty. The lesions have no definite boundary zone, but are infiltrated with cells, amongst which leucocytes are numerous. Many of the leucocytes fail to take a protoplasmic stain, but a considerable number of them show a finely granular protoplasm; no coarsely granular leucocytes have been found.

Calf 301.—Killed, when moribund, 33 days after subcutaneous inoculation with a tissue emulsion containing human bacilli found to be of variable virulence for the bovine. Numerous miliary tubercles are present; they often contain giant cells, and show less tissue destruction and more tendency to the formation of a fibrous periphery than is usual in acute lesions. Bacilli are very numerous in the lesions. Multinuclear leucocytes are fairly plentiful in the same situations; the majority of them show a definite, but not a very coarse, granulation. Some of the cells giving an oxyphil stain contain bacilli.

Calf 153.—Killed, when moribund, 39 days after subcutaneous inoculation with an emulsion of tissue containing bacilli belonging to a human virus isolated from sputum. The lesions, though resembling the acute type in their irregular outline and the absence of a well marked boundary zone, contain fewer bacilli and show a larger infiltration with wandering cells than is usual with very acute lesions. Leucocytes are numerous in the lesions; some of them have lost their granulation, but the majority are distinctly granular, and in a considerable number the granules are coarse. Bacilli are scanty, and none have been found actually being ingested by leucocytes.

Calf 191.—Killed, when moribund, 60 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at about 2½ million, derived from a human virus of high virulence for the bovine. The liver contains large tubercles in an advanced] stage of caseation and also smaller lesions, showing little or no caseation. The lesions are surrounded by a well marked cellular zone, but this shows only occasional signs of fibroid transformation. Bacilli are rather scanty. The characters of the leucocytes, and their relations to the bacilli, are identical with those found in Calf 153.

Calf 440.—Died 72 days after subcutaneous inoculation with an equine virus of high virulence for the bovine. In one of the specimens the only lesion is a small tubercle which is completely caseous in the centre and has a well marked cellular periphery ; at the periphery bacilli are numerous but none have been found within intact leucocytes ; these cells are moderately numerous, many of them being broken down. In another specimen there are several small, partially necrotic lesions with a less well-defined periphery and containing fewer bacilli ; in these lesions there is a sprinkling of leucocytes ; some of them have lost their protoplasm, whilst many are finely granular ; no coarsely granular forms have been found.

LATER LESIONS—CHRONIC.

Calf 767.—Killed 25 days after subcutaneous inoculation with 50 mg. of human bacilli possessing low virulence for the bovine. The liver contains a few minute tubercles which are surrounded by fibroblasts and in some cases contain giant cells. No bacilli have been found in the lesions, but leucocytes are moderately numerous and are generally granular.

Calf 185.—Killed 40 days after subcutaneous inoculation with ·75 cc. of an old culture belonging to a human virus of low virulence for the bovine. The liver shows several small lesions which are slightly caseous, contain many giant cells, are surrounded by fibroblasts, and in every respect are typical of the chronic, retrogressive stage. Multinuclear leucocytes are abundant in the tubercles, and in these situations are filled with rather small but well defined granules. These cells also occur in moderately large numbers throughout the liver substance, generally exhibiting a granulation, but not always such a definite granulation as in the lesions. Bacilli are extremely scanty ; those found occur within giant cells.

Calf 165.—Killed 57 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at about 4½ million, derived from a human virus of low virulence for the bovine. The liver contains typical tubercles, surrounded by fibroblasts, containing giant cells but very few bacilli, and evidently non-progressive. Leucocytes are fairly numerous in the lesions ; the majority are granular, and in the case of some the granules are rather coarse and show a tendency to escape from the cell body.

Calf 312.—Killed 90 days after subcutaneous inoculation with a porcine virus found not to possess high virulence for the bovine. The lesions present are small, not necrotic, and show little or no fibrous boundary ; a few giant cells occur, but bacilli are very rare. The main feature of the lesions is that they are infiltrated with large numbers of lymphocytes and a smaller number of leucocytes. Many of the leucocytes fail to take a protoplasmic stain ; the remainder are finely granular.

Calf 525.—Killed 90 days after subcutaneous inoculation with 50 mg. of a human virus of high virulence for the bovine. The liver shows small tubercles containing many giant cells but very little completely necrotic material ; the periphery of the lesions contains fibroblasts and large numbers of lymphocytes ; leucocytes are also present but are relatively few in number ; most of those found are of the finely granular type. No bacilli have been found. The relative scarcity of leucocytes may be associated with the absence of bacilli and the paucity of breaking down tissue.

Calf 360.—Killed 91 days after subcutaneous inoculation with 10 mg. of bovine bacilli. The liver contains a few small caseo-calcareous foci which have a dense boundary of fibroblasts and are infiltrated with a considerable number of finely granular oxyphil leucocytes. Bacilli are scanty.

Heifer 30.—Killed 115 days after subcutaneous inoculation with an emulsion of tissue containing bacilli (probably scanty in number) of bovine origin. The liver contains large caseous tubercles which are completely surrounded by a very dense fibrous zone, which is not completely avascular, but appears to be extending peripherally. Bacilli are very rare. The tubercles contain extremely large numbers of leucocytes, both internal to the fibrous zone and in the interstices of this zone. These cells are also very numerous outside the fibrous periphery. They all take a well marked oxyphil stain, and tend to have rather a coarse granulation, especially when occurring amongst the fibrous tissue.

AFTER INTRAVENOUS INOCULATION.

Calves 529, 831, 551, and 749.—These animals all died at early periods (13, 17, 21, and 24 days) after intravenous inoculation with a large dose of bacilli of human origin. The livers show a tendency to diffuse necrotic change and also contain small cellular foci, hardly definite enough to be called tubercles, in which leucoyctes occur, but not in large numbers. Bacilli are generally distributed throughout the tissues, but in all four livers there is an absence of any definite evidence that they are being attacked by the leucocytes. No bacilli have been found within leucocytes. Leucocytes are scanty on the whole, but more numerous in the 13 days' animal (Calf 529) than in the others.

Calf 305.—Killed, when moribund, 26 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 730 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. Throughout the substance of the liver minute tubercles are distributed in very large numbers. They show some tendency to caseation but the amount of tissue destruction is small, relatively to the large numbers of bacilli present. Leucocytes are found in fairly large numbers in all the lesions and take an oxyphil stain, but without marked granulation ; they occur even in the earliest lesions, where the liver cells show any sign of breaking down. But apart from the lesions leucocytes are scanty. In this case, therefore, the leucocytes appear to have been definitely attracted to the sites where the bacilli have commenced to set up degenerative change.

Calf 399.—Killed, when moribund, 34 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 700 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. The condition of the liver, as regards lesions, leucocytes, and bacilli, is identical with that of Calf 305.

Calf 319.—Killed, when moribund, 57 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at over 100 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. Throughout the liver bacilli are very numerous and have evidently multiplied freely, but they have produced very little tissue reaction and have not led to the formation of any definite tubercles. Leucocytes, oxyphil but non-granular, are scattered throughout the liver singly or in twos and threes, but none have been found in immediate contact with bacilli.

Calf 361.—Killed 76 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 41½ million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. The liver contains typical chronic, circumscribed tubercles, in which bacilli are rare. Within the lesions and at their periphery leucocytes are moderately numerous and are generally finely granular ; apart from the tubercular foci leucocytes are relatively scanty.

(4.) In the Kidneys.

The following examples are arranged in order, according to the duration of the experiment and commencing with the shortest :—

Calf 529.—Died 13 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at about 1,000 million, derived from a human virus. There is a slight degree of interstitial infiltration, but no histologically tubercular lesions have been found. Tubercle bacilli are present in scanty numbers in the interstitial tissue, and a few have been found within glomeruli. Leucocytes are in excess of the normal, but not at all numerous; they show no close relationship to the bacilli.

Calf 288.—Killed 14 days after subcutaneous inoculation with 50 mg. of bovine bacilli. There are several small lesions in the cortex and there are patches showing a slight degree of cellular infiltration between the tubules. In these situations bacilli are found, but in very small numbers. None of them have been found within leucocytes; these cells, of the ordinary finely granular variety, are present in the lesions, but only in small numbers.

Calf 831.—Died 17 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at about 1,000 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. In addition to the interstitial infiltration, similar to that described above in Calf 529, there are recognisably tubercular lesions, surrounded by a zone of lymphocytes and leucocytes and containing in their centres partially obliterated renal cells. Bacilli are numerous. The leucocytes are not very plentiful and do not appear definitely to be attacking the bacilli; some of the leucocytes are broken down.

Calf 551.—Died 21 days after intravenous inoculation with 100 mg. of culture derived from a human virus of low virulence for the bovine when inoculated subcutaneously. The only histological change is a slight degree of interstitial infiltration, similar to that found in Calf 529. Both bacilli and leucocytes are rare and there appears to be no close relationship between the two.

Calf 325.—Killed, when moribund, 24 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at about 500 million, which were of human origin and had acquired high virulence for the bovine. Numerous small, breaking down tubercles are present; they contain large numbers of bacilli and are obviously of an acutely progressive character. In the lesions the leucocytes, many of which are broken down, are numerous and are obviously attacking the bacilli.

Calf 305.—Killed, when moribund, 26 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at about 730 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. The condition is similar to that of Calf 529. There is a slight degree of interstitial infiltration, associated with scanty numbers of bacilli. The leucocytes are not numerous and do not appear to be actively attacking the bacilli.

Calf 489.—Killed, when moribund, 28 days after subcutaneous inoculation with 50 mg. of a human virus of high virulence for the bovine. The lesions are not very numerous; some of them are only slightly caseous; in others the caseation is more advanced. Bacilli are numerous in the latter lesions but rather scanty in the former. All the lesions have a broad margin of cellular infiltration, amongst which finely granular oxyphils are present but scanty. More in the interior of the older lesions no cells with oxyphil protoplasm are demonstrable but there is much nuclear débris, some of which may represent broken down leucocytes. The main feature of the specimen is that there is no evidence of recent attraction of leucocytes towards the bacilli or the tubercles. In the normal portions of the tissue leucocytes are scanty.

Calf 301.—Killed, when moribund, 33 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at about 540 million, derived from a human virus which became of increased virulence for the bovine. The tissue contains many small disseminated tubercles which are breaking down, contain numerous bacilli, and are clearly progressive in type. In some of the lesions the leucocytes are not very numerous and generally exhibit a finely granular protoplasm; in others the leucocytes are more numerous but many of them are broken down and fail to take a protoplasmic stain.

Calf 387.—Killed, when moribund, 34 days after subcutaneous inoculation with 50 mg. of a human virus of high virulence for the bovine. Small, caseous lesions are present. They have irregular margins, with no indication of demarcation, and contain fairly large numbers of bacilli. Leucocytes of the finely granular type, and giving a good oxyphil stain, are fairly abundant in the lesions. There is also a considerable sprinkling of these cells in the non-tubercular parts of the tissue. The general appearance indicates a recent immigration of leucocytes and a definite attraction of these cells to the tubercles.

Calf 121.—Killed 36 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at about 360 million, derived from a human virus of high virulence for the bovine. Two small tubercular foci have been found, containing very few bacilli and showing very little tissue destruction. Oxyphil leucocytes occur in these two situations, and in one of them there is a small group of these cells. The leucocytes are markedly granular, and some of them have shed their granules. These foci are a good example of early lesions of the chronic, and probably retrogressive, type.

Heifer 197.—Killed, when moribund, 51 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at about 85½ million, derived from a human virus of high virulence for the bovine. The tissue contains large circumscribed tubercles, circular in outline and showing progressive caseation with a slight tendency to calcification. Bacilli are scanty. Leucocytes are very abundant in the lesions. With the exception of those in the centre, many of which are broken down, the leucocytes are markedly granular, the granules, in different cells, showing differences in size and varying from the fine to the distinctly coarse.

Calf 141.—Killed 53 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at about 250,000, derived from a human virus of high virulence for the bovine. The tissue shows numerous lesions, which contain many bacilli, and are evidently progressive. In the more advanced and caseating tubercles multinuclear leucocytes are abundant and generally show a well marked granulation; in the earlier foci leucocytes are also present, but in relatively smaller numbers. The general appearance indicates a definite attraction of leucocytes to the tubercles.

Calf 319.—Killed, when moribund, 57 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at about 100 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. There is an extensive interstitial infiltration, with some patches of commencing necrosis, but the lesions are not, histologically, typical of tuberculosis. Bacilli are present in very large numbers. Oxyphil leucocytes, many of which have retained a finely granular protoplasm, are numerous in the sites which show commencing necrotic change, but are less frequent in other parts of the infiltrated areas. No bacilli have been found within leucocytes.

Calf 191.—Killed, when moribund, 60 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at 2½ million, derived from a human virus of high virulence for the bovine. The kidneys show discrete, fibro-caseous tubercles containing moderate numbers of bacilli. Though chronic in type and showing features indicative of conservative change, the lesions cannot be regarded as definitely retrogressive. They contain numerous leucocytes which are markedly granular and have a tendency to shed their granules.

Calf 122.—Killed 64 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at about 1 million, derived from a bovine virus. There are several caseo-calcareous tubercles of circular outline, bordered by fibrous tissue and containing peripherally many giant cells. Smaller tubercles, showing little or no caseation, are also present; and there are also some narrow, wedge-shaped patches of cellular infiltration. Bacilli have not been found in the last-mentioned situation and are scanty in the other lesions. Finely granular oxyphil leucocytes are scattered in large numbers throughout the older tubercular foci, except in regions of complete necrosis. Of the smaller and less advanced foci, some contain many leucocytes and others very few. In the wedge-shaped patches of infiltration leucocytes are not numerous.

Calf 128.—Killed 64 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at 110,000, derived from a bovine virus. The kidneys contain many caseating tubercles in which bacilli are rare. The lesions are extensive, irregular in outline, and probably slowly progressive. Oxyphil leucocytes are found in the lesions in large numbers and are often collected into groups within or near sites of commencing necrosis. Many of the cells are coarsely granular.

Calf 361.—Killed 76 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 41½ million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. The tissue contains small, caseous tubercles in which bacilli are scanty. The tubercles are surrounded by a well defined cellular zone. Leucocytes are rather scanty in some of the lesions, but more numerous in others. Many of them are coarsely granular.

Calf 787.—Died 81 days after subcutaneous inoculation with 50 mg. of human bacilli possessing high virulence for the bovine. There are numerous caseating lesions, rich in bacilli and obviously of the progressive type. Oxyphil leucocytes, finely granular and with a well stained protoplasm, are exceptionally numerous, both at the periphery and in the interior of the lesions. There appears to have been a recent copious immigration of these cells, which are actively attacking the bacilli.

(5.) In Lymphatic Glands.

AFTER TUBERCULIN.

Calf 135.—Killed 159 days after subcutaneous inoculation with tissue containing bacilli of human origin and of low virulence for the bovine. Tuberculin was inoculated on the right side of the neck 19 hours before the animal was killed. Rise of temperature, 2·4° C. As examples of non-tubercular glands in an infected animal which had just previously reacted to tuberculin, the three following glands were investigated. They were found to be free from bacilli and from any histological evidence of tuberculosis. (1) *Right Prescapular Gland.* Coarsely granular eosinophils occur in small numbers throughout the gland. There is a very large increase in the finely granular oxyphils, particularly in the lymph sinuses, many of which are packed full of them. They are not, however, very uniformly distributed. From the lymph sinuses the cells are seen passing into the lymphoid tissue. (2) *Left Popliteal Gland.* Coarsely granular oxyphils are present in small numbers. Finely granular oxyphils are less abundant than in the right prescapular gland, but are numerous in patches, both in the lymph sinuses and in the midst of the lymphoid tissue. (3) *A Mesenteric Gland.* The number of coarsely granular oxyphils is rather greater than in the left popliteal gland, and the number of finely granular is rather less. These observations indicate that in an infected animal inoculation with tuberculin is immediately followed by an increase in the number of finely granular oxyphils present in the lymphatic glands, the increase being observable in glands remote from the site of inoculation, though more marked in the nearest glands. The coarsely granular oxyphils, normally present in lymphatic glands, do not seem to be affected either in number or appearance.

EARLY LESIONS.

Calf 438.—Killed 24 hours after subcutaneous inoculation in the neck with 44 mg. of bovine bacilli. The prescapular gland on the same side of the neck contains a few bacilli, but no leucocytes have been found in association with them. Finely granular oxyphils are present in the gland, and in some of the lymph sinuses, particularly in the medullary portion, they are present in considerable numbers, distinctly above the normal; they are much less numerous, however, than in the prescapular gland of Calf 135, killed 19 hours after inoculation with tuberculin.

Calf 284.—Killed three days after subcutaneous noculation with 50 mg. of bovine bacilli. In the glands near to the seat of inoculation (prescapular and prepectoral) bacilli are not very numerous and are not directly associated with leucocytes, but these cells (finely granular) occur in large numbers, particularly in the medullary part of the gland and the intermediate region between medulla and cortex, whereas the bacilli are found in the cortex.

Calf 873.—Killed three days after subcutaneous inoculation with 50 mg. of human bacilli possessing low virulence for the bovine. In the nearest gland (prescapular) bacilli are moderately numerous, being freely distributed throughout the cortical part of the gland, and in some places have produced slight degenerative change in the gland substance. Finely granular leucocytes are abundant and are often collected into groups. There is no evidence, however, that they are directly attacking the bacilli. In a specimen stained by carbol-fuchsin, followed by eosin and then by methylene blue, the absence of relationship between the leucocytes and the bacilli is noteworthy. In searching the specimen with the oil-immersion lens, fields may be found containing from 5 to 20 bacilli but without a single leucocyte in view; when bacilli and leucocytes are present in the same field, the bacilli are not found either within or in contact with the leucocytes; and leucocytes very frequently occur, both singly and in groups, where there are no bacilli.

Calf 292.—Killed eight days after subcutaneous inoculation with 50 mg. of a bovine virus. In the prescapular gland, on the same side of the body, both bacilli and leucocytes are very abundant, and there are many areas of early necrotic change. The leucocytes are of the finely granular and non-granular types: the majority of them, though giving a good oxyphil stain, show no sign of granulation under the oil-immersion lens; and some of the leucocytes have lost their protoplasmic stain. Not unfrequently leucocytes are found dividing amitotically; a spherical portion of the nucleus breaks off, and gives rise to the small mononuclear oxyphil leucocyte. The leucocytes have definitely converged upon the foci where bacilli are numerous, and both those which retain and those which have lost their protoplasmic stain are found in close contact with the bacilli. In a specimen stained to show both the bacilli and the oxyphil protoplasm of the leucocytes, bacilli are found actually within the eosinophil protoplasm of the leucocytes, though they occur more frequently within other cells. In a portal gland the same condition is found as in the prescapular, the leucocytes again manifesting active phagocytosis; some of the leucocytes, though retaining their oxyphil stain, contain several bacilli; the protoplasm of the leucocytes is non-granular. In a bronchial gland the invasion of bacilli appears to be more recent, and bacilli are scantier. They are not directly associated with leucocytes. There are large masses of finely granular oxyphil leucocytes, particularly in the medulla; a few coarsely granular oxyphils are also present. In a mediastinal gland bacilli are more numerous, and in some places have produced slight lesions; there is again an absence of direct relationship between the bacilli and the leucocytes. In a popliteal gland bacilli are present but are very rare on the whole. There is, however, one small clump consisting of between 30 and 40. In this last situation there are the nuclear fragments of several leucocytes.

Calf 881.—Killed 8 days after subcutaneous inoculation with 50 mg. of human bacilli of low virulence for the bovine. In the nearest glands (prescapular and prepectoral) bacilli though freely distributed, are much less numerous than in Calf 292 and have produced less extensive lesions. Leucocytes are fairly numerous but no large groups of these cells have been found. No bacilli have been found either actually within or in immediate contact with leucocytes. A cervical gland has also been examined and been found to be in the same condition as the prescapular.

Calf 288.—Killed 14 days after subcutaneous inoculation with 50 mg. of a bovine virus. In the nearest glands (prescapular and prepectoral) bacilli are very numerous throughout, and there is advanced tissue destruction. Leucocytes, a great many of which have broken down and lost their protoplasmic stain, are vigorously attacking the bacilli and ingesting them. In a mediastinal gland the lesions, though numerous and commencing to caseate are much less advanced. They contain numerous bacilli and large numbers of oxyphils ; these cells retain their protoplasmic stain better than the corresponding cells in the preceding glands ; they are definitely attacking and ingesting the bacilli. In a popliteal gland there are earlier deposits of bacilli, in rather small numbers, in places showing slight caseous change. Leucocytes are numerous and have been definitely attracted to the tubercular foci. A few bacilli are definitely within leucocytes, but the great number are within, or attached to, cells of other types. The majority of the leucocytes give a good protoplasmic stain but show no granulation.

Calf 869.—Killed 14 days after subcutaneous inoculation with 50 mg. of a human virus of low virulence for the bovine. In the nearest glands (prescapular and prepectoral) bacilli are numerous, though less numerous than in the corresponding glands of Calf 288, and in the cortex have produced extensive tracts of commencing necrosis. In these areas leucocytes are very numerous and some are attacking and ingesting the bacilli. In a bronchial and a mediastinal gland there are a few minute lesions, in which bacilli are scanty. No distinct relationship can be observed between the bacilli and the leucocytes.

Calf 436.—Died 16 days after subcutaneous inoculation with 50 mg. of an equine virus possessing high virulence for the bovine. A mediastinal gland contains numerous small foci which contain many bacilli are commencing to caseate, and show no demarcation zone. Leucocytes are abundant in these areas, many of them being broken down. Some of the bacilli are actually within leucocytes.

LATER LESIONS—ACUTE.

Calf 849.—Died 23 days after subcutaneous inoculation with 50 mg. of human bacilli possessing high virulence for the bovine. In a mediastinal gland there are many caseous foci of the ordinary acute type and containing many bacilli. The gland is highly vascular and many vessels are present even in the caseous areas. Broken down leucocytes are numerous in the lesions, but there is no evidence of recent leucocytic invasion.

Calf 230.—Died 34 days after subcutaneous inoculation with 50 mg. of bovine bacilli. In a mediastinal gland the lesions are of the same type as in the last specimen but rather more advanced. In addition to the usual evidence of broken down leucocytes, there is some evidence of a continued leucocytic invasion, these cells (with an intact oxyphil protoplasm) being demonstrable both within the lesions and circulating in the unaffected lymph sinuses.

Calf 559.—Killed, when moribund, 39 days after subcutaneous inoculation with 50 mg. of a strain of human bacilli possessing high virulence for the bovine. The lesions in a mediastinal gland are of the typical caseous, acutely progressive type and are rather more advanced and less vascular than in the two preceding specimens. Within the older lesions nearly all the leucocytes have lost their protoplasmic stain, but at their periphery leucocytes with a good oxyphil protoplasm are fairly numerous ; and in the less affected medullary part of the gland large groups of oxyphils are sometimes found.

Calf 362.—Died 53 days after subcutaneous inoculation with 10 mg. of bovine bacilli. A popliteal gland contains bacilli in very large numbers. In the lesions which are not completely necrotic there are large aggregations of leucocytes, which are actively phagocytic.

Calf 787.—Died 81 days after subcutaneous inoculation with 50 mg. of a human virus of high virulence for the bovine. The lesions in a mediastinal gland are typical of the acute type, with irregular ramifying caseous tracts and enormous numbers of bacilli. Leucocytes are very abundant, both broken down cells and cells which retain a non-granular oxyphil protoplasm. Even in the centre of caseous areas cells giving a bright oxyphil stain are often abundant. The leucocytes are actively phagocytic.

LATER LESIONS—INTERMEDIATE.

Calf 426.—Died 29 days after subcutaneous inoculation in the neck with 200 mg. of bacilli derived from a porcine virus found in many experiments to be of low virulence for the bovine. The prescapular gland on the same side of the body is extensively affected. The lesions are of the diffuse fibro-caseous type without demarcation into discrete tubercles ; the tendency to fibrous tissue formation is much more marked than in acute lesions. Bacilli are scattered all over the tissue, and sometimes from rather large groups. There is a similar general distribution of leucocytes ; some have lost their protoplasmic stain, others are oxyphil but non-granular, and others are finely granular. Broken down leucocytes often occur near groups of bacilli. Bacilli are sometimes found within leucocytes, but more frequently within cells of other types.

Calf 216.—Killed 46 days after subcutaneous inoculation with 25 mg. of bovine bacilli. A bronchial gland shows extensive caseous tracts, with no sharp demarcation zone, and the general appearance of a slowly progressive type of lesion. Bacilli are much less numerous than in acute lesions, though by no means scanty. Leucocytes are numerous and are distributed all over the tissue, even in the caseous material. The majority take a protoplasmic stain and often exhibit, more or less distinctly, a fine granulation. The leucocytes are not actively converging upon the bacilli.

Calf 965.—Killed, when moribund, 60 days after subcutaneous inoculation with a human virus of high virulence for the bovine. A thoracic gland shows extensive caseous tracts, of the same type as in the gland of Calf 216, and containing fewer bacilli than are usually found in acute lesions. A few giant cells are present. Though there is a considerable amount of nuclear débris, there is no evidence of a recent invasion of leucocytes.

Calf 406.—Killed 91 days after subcutaneous inoculation with 10 mg. of equine bacilli belonging to a virus of relatively low virulence for the bovine. In a mesenteric gland there are many caseous tubercles with a well marked peripheral zone containing fibrous elements and giant cells. Earlier lesions, slightly caseous and containing giant cells, but without a definite demarcation zone, are also present. Tubercle bacilli are very numerous in the lesions, and have often grown into colonies. Oxyphil leucocytes are present, both non-granular and finely granular, both in the lesions and in the rest of the tissue, but are nowhere very numerous. They show no definite evidence of attacking the bacilli either at the periphery of the older lesions, where the bacilli have grown into colonies, or in the earlier lesions, where bacilli are also numerous. In the central parts of the older lesions there are many partially broken down leucocytes which contain bacilli.

Calf 474.—Killed 92 days after subcutaneous inoculation with 50 mg. of a bovine virus. A bronchial gland is extensively caseous and contains many giant cells. The lesions are evidently progressive, though there is an imperfect layer of fibroblasts at the periphery of some of them. Bacilli are moderately numerous. Leucocytes are fairly numerous in the lesions and some of them are broken down, but there is no close relationship between the bacilli present and the leucocytes.

LATER LESIONS—CHRONIC.

Calf 767.—Killed 25 days after subcutaneous inoculation with 50 mg. of a human virus of low virulence for the bovine. In the neighbouring prescapular gland there is advanced tissue destruction with caseo-fibrous degeneration. Bacilli are moderately plentiful but do not form large groups or show any sign of active multiplication. The breaking down areas are filled with enormous numbers of leucocytes. Some of them are broken down, but the great majority retain an oxyphil stain. A large proportion of the cells are granular, the size of the granules varying, in different cells, from the fine to the rather coarse. External to the areas where necrotic change has commenced, leucocytes are also abundant and show a well marked granulation. The leucocytes are actively phagocytic. In a prepectoral gland the general condition is similar to that found in the prescapular, but the lesions are less advanced and contain fewer bacilli. In a portal gland there are many minute, slightly caseous foci and a few giant cells. Bacilli are found in the caseating foci but are rare and generally only occur singly. The gland is invaded with large numbers of finely granular leucocytes ; these are plentiful throughout the tissue and are particularly abundant in the lesions ; none have been found containing bacilli. A thoracic gland is in practically the same condition as the portal ; perhaps some of the lesions are rather more advanced and the leucocytes rather fewer, but the differences are only slight.

Calf 185.—Killed 40 days after subcutaneous inoculation with ·75 cc. of an old human culture of low virulence for the bovine. The prescapular gland near the site of inoculation shows extensive caseous degeneration with advanced fibroid change in the areas which have escaped destruction. Except in completely caseous areas, where they are fairly plentiful, bacilli are rare ; some occur within giant cells but very few have been found within leucocytes. Leucocytes, of the finely granular type, are numerous in all parts of the section where the tissue is not broken down. There is also a collection of these cells, many of them in fragments, at the margin between the cellular elements and the caseous material.

Calf 508.—Killed, when moribund, 73 days after subcutaneous inoculation with 50 mg. of an equine virus of high virulence for the bovine. In a popliteal gland there are small, caseous tubercles containing giant cells and surrounded by fibroblasts. The lesions contain bacilli in rather large numbers. There is a sprinkling of leucocytes, most noticeable at the periphery of the lesions, but none have been found in contact with bacilli.

Calf 273.—Killed 89 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at 11 million, derived from a strain of human bacilli of low virulence for the bovine. In a thoracic gland many tubercles of various sizes are present. The largest are in an advanced stage of caseation and contain a little calcareous deposit, whilst the smaller are only slightly caseous. The tubercles, though containing a considerable amount of fibrous elements at their periphery and having the general appearance of chronic rather than acute lesions, appear to be slowly progressive and becoming confluent. Bacilli are scanty. Except where completely necrotic, the lesions are infiltrated by large numbers of leucocytes.

Calf 993.—Killed 88 days after subcutaneous inoculation with 50 mg. of a human virus possessing low or intermediate virulence for the bovine. In a thoracic gland there are small lesions which have a completely caseous centre and are surrounded by a well defined ring of fibrous tissue. Bacilli are scanty and are only found within the caseous material. At the margin of the lesions and slightly encroaching on the caseous substance, leucocytes are numerous.

Calf 116.—Killed 92 days after the commencement of a feeding experiment (sucking a cow with tuberculosis of the udder produced by bovine bacilli). In a mesenteric gland large numbers of giant cells occur both singly and in groups in various parts of the tissue. There are also some tracts of calcareous deposit surrounded by a zone of the epithelioid type of tissue and without any intermediate caseous area. Bacilli are present in many of the giant cells and also in the calcareous deposits but none have been found within leucocytes. Leucocytes are fairly numerous throughout the gland and are not specially aggregated in the tubercular lesions. They are almost all granular, the granules being more frequently coarse than fine.

AFTER INTRAVENOUS INOCULATION.

Calf 529.—Died 13 days after intravenous inoculation with a tissue emulsion containing bacilli of human origin, estimated at 1,000 million. A sternal gland is congested but shows no histological lesions. Bacilli are present in many parts of the tissue and often occur in small groups. Leucocytes are scanty and show no close relationship to the bacilli.

Calf 831.—Died 17 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 1,000 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. In an iliac gland the only lesions are a large number of minute foci consisting of cell-fusions and sometimes showing definite giant cell formation. Bacilli occur in all these situations and are scattered about the rest of the gland singly or in small groups. Leucocytes are not numerous and nowhere form large groups, but these cells are sometimes found in direct contact with bacilli.

Calf 89.—Killed, when moribund, 23 days after intravenous inoculation with a tissue emulsion containing bacilli, estimated at about 300,000, derived from a human virus isolated from sputum. A mediastinal and a thoracic gland show typical acute infection with progressive tissue destruction, bacilli being distributed in large numbers all over the tissue. Leucocytes are abundant in the areas showing necrotic change, but not frequent outside these situations. The majority give an oxyphil stain but are non-granular. They are actively phagocytic.

Calf 749.—Killed, when moribund, 24 days after intravenous inoculation with 46 mg. of a human virus possessing low virulence for the bovine when inoculated subcutaneously. In a bronchial gland there are very large numbers of bacilli throughout the tissue, but there are no definite histological tubercles, and the general amount of tissue damage is only slight. Leucocytes are present in large numbers and are specially collected in places where bacilli are abundant. The leucocytes are actively phagocytic.

Calf 305.—Killed, when moribund, 26 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at about 700 million, derived from a human virus of low virulence when inoculated subcutaneously. In a bronchial gland the cortex is riddled with semi-necrotic, confluent areas, and there are also caseating lesions, though less numerous, in the medulla. Bacilli occur in all the lesions in moderate numbers. Coarse eosinophils are present in small numbers in the unaffected parts of the gland ; ordinary leucocytes are abundant everywhere ; in the necrotic areas large numbers of these cells have lost their protoplasmic stain ; elsewhere the leucocytes give a good, non-granular or finely granular, oxyphil reaction. The leucocytes, particularly those which have lost their protoplasmic stain, are in direct contact with the bacilli.

Calf 399.—Killed, when moribund, 34 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at about 700 million, derived from a human virus of low virulence for the bovine when inoculated subcutaneously. A bronchial gland closely resembles the bronchial gland of Calf 749 as regards the relatively slight amount of tissue damage, the number and distribution of bacilli, the presence of large numbers of leucocytes, and the active phagocytosis of these cells.

Calf 101.—Killed, when moribund, 41 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 48,000, belonging to a human virus of high virulence for the bovine. An iliac, a bronchial, and a coeliac gland have been examined. They all exhibit lesions of the same characters, viz., tubercles in an early stage of caseation and sometimes containing giant cells, and also isolated giant cells situated amongst normal tissue. Bacilli are present in all the lesions but are not very numerous. In all three glands coarsely granular oxyphils occur ; they are present in the non-affected parts of the tissue and are quite as numerous as is usually the case in normal glands. In the iliac and the coeliac glands finely granular or non-granular oxyphils are rare ; there is thus a notable absence of leucocytic infiltration. In the bronchial gland these oxyphils are more numerous in some of the lymph channels, but in the tubercular foci there is again only a slight degree of leucocytic infiltration.

Calf 555.—Killed, when moribund, 58 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at 150 million, derived from a human strain possessing high virulence for the bovine. A lumbar gland shows diffuse caseation of the type usually met with in highly acute infections and contains very large numbers of bacilli. Leucocytes are distributed in large numbers throughout the tissue, but the majority of them are broken down, and very few of them take an oxyphil stain. There is therefore no evidence of recent leucocytic invasion.

Calf 361.—Killed 76 days after intravenous inoculation with an emulsion of tissue containing bacilli, estimated at 41½ millions, derived from a human virus of low virulence for the bovine. A peribronchial gland is very extensively caseous and contains numerous giant cells. Bacilli are present in all the lesions, but are not abundant ; most of them are found in completely necrotic areas, but a few occur nearer to the periphery of the lesions and are in contact with broken-down cells, some of which may have been leucocytes. There is no sign of recent leucocytic invasion.

(6.) In Mammary Glands.

Cow 4.—Killed 34 days after intramammary inoculation with an emulsion of tissue containing bovine bacilli. The udder shows acute, progressive infection with rapid tissue destruction, oedema of the surrounding parts and great multiplication of bacilli. Leucocytes are very numerous in the lesions and, except in situations where all the cellular material is breaking down, give a good, non-granular, oxyphil stain. They are actively phagocytic.

Cow 75.—Killed, when moribund, 34 days after intramammary inoculation with an emulsion of tissue containing bacilli, estimated at 38 million, derived from a human virus of high virulence for the bovine. The lesions closely resemble those found in Cow 4, but are perhaps not quite so acute. The relations of bacilli and leucocytes are the same as in Cow 4.

Cow 68.—Killed 48 days after intramammary inoculation with an emulsion of tissue containing about 30 million bovine bacilli. The udder shows progressive tuberculosis of the caseous type, with a tendency to calcification. The lesions are less acute in character than those found in the two preceding animals, and bacilli are much less numerous. Oxyphil leucocytes occur both within the lesions and in the surrounding tissues ; in some lesions they are less numerous than in others, but on the whole they are abundant. They are usually of the finely granular type and show a more distinct granulation than the leucocytes in the more acutely infected udders.

Heifer 249.—Killed, when moribund, 54 days after subcutaneous inoculation with 50 mg. of a human virus possessing high virulence for the bovine. The udder contains some small tubercles which are slightly caseous and contain giant cells. Bacilli are very rare. Within the tubercles leucocytes are rather scanty and have lost their protoplasmic stain. In the neighbourhood of these lesions there is a good deal of cellular infiltration between the mammary nodules. My first impression was that leucocytes were rare in these situations, but on searching further specimens I have found them in considerable numbers. They take a good oxyphil stain and are generally non-granular. Mononuclear forms are present. Some of the cells are dividing amitotically and show mononuclear forms in process of formation.

Cow 3.—Killed 63 days after intramammary inoculation with a culture of human bacilli possessing low virulence for the bovine. The udder shows a chronic slowly progressive tuberculosis. The majority of the lesions are caseous and have a well marked peripheral zone containing numerous fibroblasts, but there are also earlier lesions, and the disease has made its way into one of the non-inoculated quarters. Bacilli are present in most of the tubercles and in some are numerous. Oxyphils, both non-granular and finely granular, are abundant both at the periphery and in the interior of the lesions. They are also abundant within the gland tubules. In a specimen stained with carbol-fuschin followed by eosin and then by methylene blue no bacilli are demonstratable within any of the leucocytes.

Heifer 251.—Killed 138 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at 3000 million, derived from a human virus of high virulence for the bovine. The udder shows early and apparently progressive tuberculosis. There are many patches of early caseation. Both in these and within the alveoli of the surrounding tissue, oxyphil leucocytes are very numerous ; they stain well and show only a slight granulation. These cells are also numerous in patches of interalveolar infiltration which have not become caseous. Bacilli are scanty, but occur both within the tubercles and in the earlier areas of infiltration.

Heifer 233.—Killed 181 days after subcutaneous inoculation with an emulsion of tissue containing bacilli, estimated at 53½ million, derived from a human virus of high virulence for the bovine. The udder shows extensive inflammatory change, probably non-tubercular in origin, and in one of the quarters there are some caseous lesions and giant cells. Tubercle bacilli are present in these last situations but are very rare. There is a considerable sprinkling of oxyphils, with a definite granulation, at the margin of the lesions which are definitely tubercular ; within the caseous areas these cells are rarer but a few leucocytes which have lost their protoplasmic stain are present. In the surrounding tissue both coarse and finely granular oxyphils are present in considerable numbers.

Cow 74.—Died 299 days after an intramammary inoculation with an emulsion of tissue containing bovine bacilli. The udder shows a very slowly progressive tuberculosis in which the amount of tissue destruction is small relatively to the enormous number of bacilli present. Broken down leucocytes are numerous within the lesions and there are many cells, probably leucocytes though giving no oxyphil stain, which contain bacilli. No bacilli have been found within leucocytes possessing an oxyphil protoplasm. There is no sign of a recent leucocytic invasion into the more advanced tubercular areas. In the non-inoculated quarters oxyphils, with a rather indistinct granulation, are present in small numbers in the interstitial tissue between the glandular tubules, but are not closely related to the bacilli, which occur, in small numbers, lying free in the interstitial tissue or between the fat cells. In other situations the granulation of the leucocytes is more definite, particularly at the margins of the larger tubercular foci and also in the neighbourhood of the earlier lesions, where there is only slight evidence of tissue degeneration.

Cow 172.—Killed 392 days after an intramammary inoculation with an emulsion of tissue containing about 30 million bacilli of bovine origin. The udder shows discrete tubercles of the ordinary chronic type, most of the lesions being centrally caseous and surrounded by a fibrous zone, though earlier caseating areas are also present. Bacilli are scanty. In the centre of some of the tubercles, oxyphils are numerous and are of the finely granular variety ; the leucocytes which occur in the peripheral zone and in the neighbouring intertubular tissue tend to assume a coarser granulation. In the tissue remote from the lesions oxyphils are scanty.

II. IN SWINE.

EARLY LESIONS.

Pig 144.—Killed 24 hours after subcutaneous inoculation in the ear with 20 mg. of a bovine virus. In a cervical gland near to the site of inoculation bacilli are found distributed throughout the specimen in small numbers. Leucocytes are very numerous throughout the gland, but as the gland contains many small deposits of dirt, the presence of these cells in large numbers may not be entirely due to the tubercle bacilli. But wherever bacilli are present there are generally many leucocytes near at hand and many leucocytes have been found with bacilli inside them; generally not more than one or two bacilli are found within a cell, but a few leucocytes have been found which are crowded with bacilli. There is therefore clear evidence that the leucocytes are actively attacking the bacilli. This is particularly noteworthy as the bacilli must be very recent arrivals and have not produced any visible breaking down of the tissue. The leucocytes are strongly oxyphil; the greater number of them are of the usual coarsely granular type met with in normal glands, but the ordinary multinuclears also show a well marked granulation; their granules are more readily dissolved than those of the former class; the leucocytes which contain bacilli are all of the latter type.

Pig 146.—Killed three days after subcutaneous inoculation in the ear with 20 mg. of a bovine virus. In a cervical gland near the site of inoculation bacilli are much more numerous than in the 24 hours' specimen; they are distributed freely all over the tissue and have produced early necrotic change. The leucocytes are also greatly increased in number. Fewer of them are coarsely granular, and a great many of them are breaking down. The leucocytes form the largest aggregations in the situations where bacilli are most numerous; they are actively phagocytic. Bacilli are frequently found both within leucocytes which retain their protoplasmic stain and within breaking down leucocytes. Some of the leucocytes are dividing amitotically.

Pig 148.—Killed five days after subcutaneous inoculation in the ear with 20 mg. of bovine bacilli. In a cervical gland near the site of inoculation bacilli are more numerous than in the three days' gland and necrotic change is rather more advanced. The leucocytes, though many of them are broken down, are still phagocytic; perhaps 1 in 50 or 1 in 100 contain bacilli.* The granulation of the leucocytes within or near the older lesions is less distinct than in the three days' specimen, but outside these areas cells are plentiful which have a well marked and sometimes a distinctly coarse granulation. The nuclei of many of the leucocytes are swollen, and some are dividing amitotically. In the lungs bacilli are very rare and are not associated with any definite tubercle formation; leucocytes are fairly numerous, but none have been found containing bacilli.

Pig 142.—Killed eight days after subcutaneous inoculation in the ear with 20 mg. of bovine bacilli. In a neighbouring cervical gland both leucocytes and bacilli are again greatly increased in numbers. The lesions are more advanced and are packed with multinuclear leucocytes and their remains. Both in these lesions and in the lymph channels leucocytes containing bacilli are frequently found. Coarsely granulars are relatively rare, but are still found in the unaffected parts of the tissue. In the spleen bacilli are rare; none have been found within leucocytes, but a few leucocytes are generally present in their neighbourhood. In the liver very few bacilli have been found. These occur within minute early tubercles, each of which contains several finely granular leucocytes; none of the bacilli found are actually within leucocytes. In the lungs there are numerous deposits of bacilli which have given rise to early tubercles; in all these situations multinuclear leucocytes, only slightly granular, are abundant; a few bacilli have been found within leucocytes, but the majority of the bacilli are within, or attached to, cells of other types. The leucocytes, therefore, though definitely attracted to the lesions, are not vigorously ingesting the bacilli.

Pig 150.—Killed 14 days after subcutaneous inoculation in the ear with 20 mg. of bovine bacilli. A cervical gland near the site of inoculation is extensively caseous and is swarming with bacilli. Leucocytes are also extremely abundant and appear to be rapidly breaking down. Bacilli are present in many leucocytes, but not in many which have retained a definite granulation. Coarsely granular leucocytes are still to be found. In a portal gland there are many early lesions, which are slightly caseous but contain very few bacilli; coarsely granular oxyphils are abundant throughout the gland; multinuclear leucocytes with no definite granules are also present; their number is not very great, but more occur within the lesions than elsewhere. None of the leucocytes have been found to contain bacilli. A bronchial gland has been found to be in a similar condition to the portal gland. The lungs are packed with early tubercles which are much more numerous and rather larger than in the eight days' specimen. In proportion to their size, the lesions do not contain, individually, more bacilli than those found in the eight days' lung. Leucocytes are plentiful and have invaded the tubercles in large numbers. They almost all give a good oxyphil stain. The great majority are non-granular or only finely granular, but coarsely granular cells are also present and may be readily found at the periphery of the lesions, rarely in the interior. Many of the leucocytes are dividing amitotically. Only a few leucocytes contain bacilli. The early lesions found in the liver are in almost the same condition as those in the eight days' specimen but slightly more advanced.

LATER LESIONS—ACUTE.

Pig 94.—Killed 61 days after subcutaneous inoculation with 1 mg. of bovine culture. The lungs are in a transitional stage between miliary tuberculosis and commencing generalised consolidation. Bacilli are numerous throughout the tissue. Leucocytes are also numerous, particularly in the breaking down foci. They are finely granular or non-granular and sometimes, though not frequently, contain bacilli. The liver shows a diffuse tubercular infiltration, the lesions not being separable into discrete tubercles. Bacilli are present, though not very abundant, in all the lesions. Oxyphil leucocytes, nearly all of the finely granular or non-granular types, are numerous in all the lesions; relatively few of them are disintegrated or have lost their protoplasmic stain; some of them show nuclear division; both amitotic and mitotic forms of division have been found. No bacilli have been found within leucocytes. A mesenteric gland shows extensive caseous change, with very large numbers of bacilli. Multinuclear leucocytes are abundant, but the majority of them are disintegrated. Some of the leucocytes contain bacilli.

Pig 92.—Killed, when moribund, 137 days after feeding with tuberculous milk estimated to contain 10 million bacilli. The specimen of lung is extensively caseous and contains large numbers of bacilli. The lesions are confluent, obviously progressive, and show no sign of a limiting zone. Typical coarsely granular eosinophil cells are numerous and are often massed together in groups. These groups occur at the margin of consolidated areas but not in situations where there is advanced necrotic change. These cells vary in size, many being twice as large as the ordinary finely granular multinuclear cell, but others no larger than the latter type of cell. The nuclei of the coarsely granular cells are either circular in outline, or oval, or slightly indented; some of them take a deep blue stain, like the nuclei of the finely granulars, but many of them only take a pale blue stain; the pale nuclei are generally larger than the deeply stained nuclei. Finely granular or non-granular leucocytes are also numerous, particularly where necrosis is commencing or advanced; they have a weaker affinity for eosin that the coarsely granulars, and many of those which retain a protoplasmic stain show no granulation. The bacilli have no relation to the coarsely granulars, but the cells of the other type have evidently been attracted to the bacilli, and sometimes bacilli are found within cells retaining a faint oxyphil stain. The liver is crowded with miliary tubercles in an early stage of caseation; from about two or three to about a dozen bacilli are visible in each. There is an infiltration of coarsely granular leucocytes throughout the interlobular tissue. Within the tubercles these cells are rare, but ordinary leucocytes, many of which have lost their protoplasmic stain, are common.

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* This was my general impression on examining the section. A count was afterwards made and the percentage of phagocytic leucocytes were found to be rather higher.

LATER LESIONS—LESS ACUTE AND CHRONIC.

Pig 26.—Killed 37 days after feeding with tuberculous milk containing 10 million bacilli. The lungs contain many small tubercles which are isolated, have a well marked boundary zone, contain very few bacilli, and are filled with dense masses of multinuclear leucocytes; these cells give a good oxyphil stain but show no definite granulation, either in the interior or at the periphery of the lesions. Two mesenteric glands examined show extensive and partially caseous tubercular areas, but do not contain many bacilli. Leucocytes are collected in enormous numbers in the interior of the lesions and are also abundant at their periphery; many are also in the surrounding tissue. They are mostly of the finely granular and non-granular type. In an ileo-coecal gland the only differences observed are that bacilli are rather more numerous and the leucocytes external to the lesions are more definitely granular.

Pig 18.—Killed 39 days after feeding with tuberculous milk containing one million bacilli. As in Pig 26, the lungs contain small tubercles of the retrogressive type which contain very few bacilli but are crowded with multinuclear leucocytes. A mesenteric and two ileo-colic glands show advanced caseation but do not contain many bacilli. As in the glands of Pig 26 the interiors of the lesions are crowded with finely granular or non-granular oxyphils; external to the lesions coarsely granulars are fairly numerous.

Pig 23.—Killed 65 days after subcutaneous inoculation with 50 mg. of a human virus of low virulence for the bovine. The lungs show marked thickening of the alveolar walls and contain numerous tubercles. These tubercles are slightly caseous in the centre and have a broad cellular zone containing many fibroblasts. Bacilli are rare. The lesions though obviously of a chronic type are tending to become confluent. Coarsely granular oxyphils are scattered all over the tissue and are numerous in the lesions, where non-granular oxyphils also occur in equally large numbers. In the liver, which contains chronic tubercles possessing very few bacilli, coarsely granular oxyphil leucocytes are found in moderate numbers outside the lesions; within the lesions leucocytes are aggregated into small groups and are of the finely granular type, though a few coarsely granulars are also present.

Pig 21.—Killed 98 days after subcutaneous inoculation with 1 mg. of a human virus of low virulence for the bovine. Specimens from two parts of the lungs show groups of circumscribed, caseous, and obviously retrogressive tubercles. They contain in or near their centres large numbers of finely granular multinuclear leucocytes; and at their periphery, which is densely fibrous, coarsely granular leucocytes are found. No bacilli have been found.

Pig 126.—Killed 114 days after subcutaneous inoculation with 1 mg. of culture of a porcine virus found to be of low, or relatively low, virulence for the bovine. Some isolated caseous tubercles are present in the lungs. They are of the chronic type and are completely surrounded by fibroblasts. At the periphery of the caseous material there is a dense zone consisting entirely of finely granular and non-granular oxyphil leucocytes. Amongst the fibrous elements the leucocytes show a stronger granulation but the granules are not definitely coarse.

Pig 80.—Killed 131 days after feeding with an emulsion of tissue containing bacilli of bovine origin estimated to number 10 million. The lung tissue is to a large extent replaced by irregular caseous areas which are obviously spreading. The alveoli bordering upon them are filled with desquamated epithelium. In the caseous areas there are many broken down leucocytes, and in the outer portion of the consolidated areas there are groups of multinuclear leucocytes which retain an oxyphil stain. Bacilli are distributed throughout the lesions but are scanty.

Pig 2.—Killed 160 days after feeding with tuberculous milk. The lungs contain large caseous tubercles which are surrounded by a broad fibrous zone. Bacilli are very rare. The most striking feature about the tubercles is that they are filled with enormous masses of multinuclear leucocytes. These cells occupy the centre as well as the more peripheral areas and are of the finely granular or non-granular type.

III.—IN RABBITS.

(1.) In Rapidly Fatal Infections.

The following examples are taken from a series of rabbits killed at short intervals after intravenous inoculation with 1 mg. of bacilli possessing high virulence for the bovine. A bovine virus was employed for half of the rabbits and a human virus, which proved to be equally virulent, for the other half. These experiments are reported in the Appendix to the Second Interim Report, Vol. IV., p. 25.

AFTER TWENTY-FOUR HOURS.

In the lungs tubercle bacilli are distributed, singly and in small groups, in numerous sites throughout the alveolar walls. Oxyphil leucocytes, generally multinuclear, and with a well-marked, coarse granulation, are found in fairly large numbers throughout the tissue and are aggregated into groups in the localities where groups of bacilli are found. Though the leucocytes are definitely attracted to these situations, it is only rarely that bacilli are found actually within leucocytes. Amongst some of the larger groups of bacilli, disintegrated leucocytes are found. No leucocytes have been observed showing evidence of nuclear division. In the liver there are a few minute deposits of bacilli; in association with these there is generally a small group of coarsely granular leucocytes, but very few bacilli have been found actually within leucocytes.

AFTER THREE DAYS.

In the lungs the lesions are rather larger than in the 24 hours' specimens and some of them contain more bacilli. The main difference noted in the leucocytes is that more of those present in the lesions have lost their granular protoplasm. Bacilli are occasionally found within leucocytes but much more frequently occur within cells of other types. In the liver there are several small groups of bacilli which are surrounded by leucocytes with a well marked coarse granulation; some of the bacilli are actually within these leucocytes. In one specimen of kidney a few bacilli have been found in the intertubular tissue, but these are not associated with leucocytes.

AFTER FIVE DAYS.

In the lungs the bacilli are much more numerous than in the three days' specimens and the lesions are rather more advanced. Leucocytes are numerous in the lesions and many show a well marked coarse granulation, cells with this character being more numerous at the periphery than at the centre of the lesions. Many of the leucocytes, including a considerable number which retain well marked granules, contain bacilli. This is well shown in specimens stained by carbol-fuchsin followed by orange and then by methylene blue. In the liver there are numerous small foci containing bacilli in small or moderate numbers. In these lesions leucocytes with a well marked granulation are present but are not more numerous than in the three days' specimens. In the tissue as a whole leucocytes are not very numerous, and though they are attracted towards the definitely tubercular foci, they are not found in association with isolated bacilli which occur elsewhere. A few bacilli have been found within leucocytes.

AFTER TEN DAYS.

The tubercles are more numerous and many of them are larger, but the majority have not begun to caseate. There is again a large increase in the number of bacilli. Leucocytes are very numerous in the tubercles and are also much more abundant in the rest of the tissue than in previous specimens. Some of the leucocytes have lost their granules, but the greater number of them are definitely granular; the granules are rather large and have a marked tendency to escape from the cell body. The leucocytes are definitely attracted to the lesions and are actively phagocytic. In many of the lesions perhaps as many as 1 in 20 of the leucocytes contain bacilli within them; and, in addition, bacilli are often found in a confused mass of loose granules and leucocytic nuclei. The liver shows a diffuse tubercular infiltration with commencing necrotic change. Bacilli are numerous. Leucocytes, though moderately numerous, are much less abundant than in the lungs. They give a good granular stain. Some of the lesions contain many leucocytes; others very few. A few of the leucocytes contain bacilli. The kidneys on the whole are only slightly affected, but in one of the specimens there is a fairly large lesion containing a moderate number of bacilli and many leucocytes, some of which are in close contact with the bacilli.

AFTER FIFTEEN DAYS.

In the lungs the tubercles are more numerous, more necrotic, and larger than in the 10 days' specimens. The bacilli are again increased in numbers and are more generally distributed throughout the tissue. The tissue is swarming with oxyphil leucocytes which have a well marked coarse granulation. These cells have a tendency to shed their granules, particularly within the tubercles, where confused masses of granules, bacilli, and nuclei are found. Bacilli are not only numerous within these confused groups of leucocytes, but are also found within isolated, coarsely granular cells which have not shed their granules. Many of the leucocytes show signs of amitotic division. In the liver the lesions are of the same type as in the 10 days' specimens but rather larger, and bacilli are more numerous. There is no increase in the number of leucocytes, compared with the previous specimens, but more of the leucocytes present have lost their granules. A few of the leucocytes are in immediate contact with, or contain, bacilli. In the kidneys bacilli are found more frequently than in the 10 days' specimens and occur both in the interstitial tissue and in the glomeruli. They have not produced extensive lesions. Leucocytes are scanty and are not definitely associated with the bacilli.

AFTER EIGHTEEN TO TWENTY-FOUR DAYS.

The remainder of the animals used in this experiment died in from eighteen to twenty-four days after inoculation. In the lungs of these animals rapidly progressive necrotic change is found, with continued multiplication of bacilli. The leucocytes retaining a granular stain are still very abundant in the 18 days' specimen, but after this period they show a gradual falling off. In the 24 days' specimen the leucocytes in the interior of the lesions are nearly all broken up, though loose granules are still present, and in places external to the necrotic areas intact granular leucocytes are found in moderate numbers. In the livers and kidneys no appreciable differences from the 15 days' specimens have been observed as regards the relations of leucocytes and bacilli.

(2.) In More Chronic Infections.

Rabbits 1567–71.—These rabbits were inoculated intravenously, each with 1 mg. of a virus of relatively low virulence (H 90. I.P.) and were killed, respectively, 24 hours, 3 days, 5 days, 8 days, and 14 days after inoculation. Compared with the rabbits inoculated intravenously with more virulent material, the number of disseminated bacilli is smaller and there is less tissue destruction. Corresponding with these differences, the leucocytosis is less abundant. The percentage of leucocytes containing bacilli is small, but bacilli are found within intact leucocytes rather more frequently, in proportion to the number of bacilli, than in the more acute infections.

Rabbits 1572–76.—These rabbits were inoculated subcutaneously, each with 50 mg. of a virus of relatively low virulence, and were killed, respectively, 24 hours, 3 days, 6 days, 8 days, and 14 days after inoculation. Apart from the local lesions, no microscopic evidence of dissemination has been found in the specimens examined. The limitation of bacilli to the local lesions is largely attributable to the activity of the leucocytes which have converged upon the site of inoculation and formed a dense barrier. In the central areas the leucocytes are crowded with bacilli but rapidly breaking down. In all the specimens leucocytes are numerous at the periphery of the lesions, though the leucocytic invasion appears to be falling off in intensity towards the end of the second week.

Rabbits 1542–46.—These rabbits were inoculated subcutaneously, each with 50 mg. of a bovine virus of high virulence, and were killed, respectively, 24 hours, 3 days, 6 days, 8 days, and 14 days after inoculation. Only slight and occasional microscopic evidence of dissemination has been found in the specimens examined. As in the series above (Rabbits 1572–76) inoculated with less virulent material, the inoculated material is densely surrounded with leucocytes, which have limited its dissemination. The general condition of the leucocytes and their relations to bacilli are the same in the two series, and in both there is a diminution of the local leucocytosis towards the end of the second week.

Rabbit 219.—Died 69 days after intraperitoneal inoculation with 50 mg. of human bacilli possessing low virulence for the bovine. The lungs are in a condition of patchy, caseous consolidation. Tubercle bacilli are present in all parts of the tissue and are numerous in some of the caseous areas. The whole of the tissue is abundantly infiltrated with leucocytes possessing a well marked coarse granulation. These cells, though numerous in the lesions, are not specially congregated round the bacilli; and bacilli are only rarely found in immediate contact with them.

Rabbit 217.—Killed 90 days after intraperitoneal inoculation with 1 mg. of the same culture as Rabbit 219. Beneath the pleural surface of the lung there is an irregularly defined tubercle, slightly caseous, abundantly infiltrated with cells, and containing very few bacilli. Coarsely granular leucocytes, though showing no immediate relationship to the bacilli, are numerous in the lesion and also in the rest of the tissue.

Rabbit 231.—Killed 90 days after intraperitoneal inoculation with 1 mg. of culture isolated from a strain of human bacilli showing low virulence for the bovine. The specimen of lung is in a semi-solid condition owing to the presence of innumerable tubercles. These are centrally caseous, irregular in outline, and frequently confluent. They are evidently progressive, though surrounded by an abundant cellular infiltration. In the majority of the foci, bacilli are scanty, but in a few of the older lesions they have grown into groups. Leucocytes with a well marked granulation are numerous in all parts of the tissue and form dense masses in the lesions. In the centres of the older lesions, where bacilli are numerous, the leucocytes are broken down. Only a few of the leucocytes contain bacilli. The liver exhibits a large number of minute tubercles, These are not caseous but exhibit cell fusions and sometimes giant cells. Granular leucocytes are present at the periphery of the lesions but are not numerous ; in the interior there are a few leucocytes which have lost their protoplasmic stain. No bacilli have been found.

Rabbit 238.—Killed 90 days after intraperitoneal inoculation with 1 mg. of culture. In the lungs there are slightly caseous patches of consolidation beneath the pleura. Bacilli are scanty. The consolidated areas are infiltrated with extremely large numbers of granular leucocytes.

Rabbit 72.—Died 179 days after intravenous inoculation with 1 mg. of a human virus possessing low virulence for the bovine. The specimen of lung is almost completely solidified. It shows large, ramifying tracts of completely necrotic material, with no demarcation into definite tubercles. Irregular patches of less advanced caseation are also present. Bacilli are numerous everywhere, though less abundant than in advanced lesions produced by intravenous inoculation of more virulent viruses. The whole of the tissue is densely infiltrated with leucocytes showing a well marked granulation. These cells are aggregated round the collections of bacilli, but where the cells occur singly relatively few are found with bacilli actually within them. The liver contains many minute, non-caseous or only slightly caseous tubercles which do not contain many bacilli. At the periphery of these lesions a few granular oxyphils are present, and a few leucocytes which have lost their protoplasmic stain are found in the interior. This condition is in marked contrast to the abundance of leucocytes found in the lungs. The kidneys contain large caseating lesions which are abundantly infiltrated with granular leucocytes. Bacilli are rather scanty on the whole, but in some places in the interior of the lesions they are collected into groups, which are being vigorously attacked by the leucocytes.

(3.) After Inoculation with Killed Bacilli.

Rabbit 1195.—Killed 21 days after intravenous inoculation with 50 mg. of dead culture from a human virus of low virulence for the bovine. In the lungs bacilli are scanty and have caused little more than slight generalised thickening of the alveolar walls. In a few places there are small collections of bacilli, many of which are fragmentary. Leucocytes are scanty and are not found in association with the bacilli. In the liver bacilli are scattered about the tissue much more abundantly than in the lungs, and have produced many small lesions, in which leucocytes are rare. In the spleen bacilli are numerous in all parts of the tissue and often occur in small groups. They have caused very little damage to the tissue, but are often surrounded by groups of granular leucocytes, some of which actually contain bacilli ; many of these cells are broken up.

Rabbit 1283.—Killed 29 days after intravenous inoculation with 50 mg. of dead bovine bacilli. Throughout the lungs the alveolar walls are very greatly thickened and many patches are completely solidified. The places where bacilli are demonstrable are generally the centre of small areas of slight caseation. These foci are characteristic of tubercles of the more chronic type. In the lesions where bacilli are very scanty and occur singly leucocytes are also scanty and are not in close contact with the bacilli ; but where small clumps of bacilli have survived they are surrounded by a dense cluster of granular leucocytes. In the liver single bacilli or fragments of bacilli are distributed throughout the tissue ; they generally occur within giant cells. Leucocytes are scanty and show no direct relationship to most of the lesions, but a few isolated leucocytes contain bacilli. In the spleen there are no tubercles or giant cells, but in a few places small groups of bacilli, often fragmentary, are present. These are generally associated with small groups of oxyphil leucocytes. The nuclei of many of the leucocytes are fragmentary.

Rabbit 1284.—Killed 29 days after intravenous inoculation with 10 mg. of dead bovine bacilli. Bacilli occur in many parts of the lungs and have produced caseous tubercles of the same characters as those in the preceding animal. Bacilli are generally rare in the lesions, but occasionally a small clump occurs. Leucocytes are not numerous and are rarely found in direct contact with bacilli. In the liver bacilli are very scanty and are not associated with leucocytes. The spleen is identical with that of Rabbit 1283.

Rabbit 488.—Killed 63 days after intravenous and intraperitoneal inoculation with cultures of bovine bacilli amounting in all to 140 mg. The lungs contain numerous deposits of bacilli, some of them consisting of clumps of very considerable size. These deposits have given rise to well formed tubercles of the caseous and circumscribed type. Granular leucocytes are abundant at the periphery of the lesions and in the interior form dense masses completely enveloping the bacilli. In the specimen of liver there is a caseous tubercle with a broad cellular zone, amongst which there are many oxyphils. No bacilli have been found in this specimen.

B.—THE SIGNIFICANCE OF LEUCOCYTIC PHAGOCYTOSIS.

Owing to the clinical importance sometimes attributed to the action of leucocytes upon tubercle bacilli, I have thought it might be useful to supplement the preceding observations upon leucocytes by a series of counts recording the amount of leucocytic phagocytosis actually observed in sections of tissues. The statistics thus provided enable me to supplement and, where necessary, correct the general results regarding leucocytic activity which I have collected from my histological reports on the examination of a very large number of specimens.

Before making a count the whole specimen is examined, and a typical portion of it is selected as the area to be estimated. Then, with the aid of the mechanical stage, 1,000 leucocytes are counted ; at the same time records are made of the number of bacilli found within the area occupied by these leucocytes, of the number of these bacilli contained within leucocytes, and of the number of leucocytes containing these bacilli. The only leucocytes counted are those the protoplasm of which takes on a stain with eosin, *i.e.*, the leucocytes which were not disintegrated when the tissue was removed from the body and fixed. The amount of phagocytosis recorded is therefore what I term "active" phagocytosis, *i.e.*, it is based on an estimate of the proportion of intact leucocytes which actually contained bacilli at the time when the experiment was terminated.

The method of staining is as follows. First, the section, fixed on a coverslip, is stained in carbol-fuchsin with steam gently rising for 20 minutes. The excess of carbol-fuchsin is washed out with methylated spirit until no more stain comes away. The section, after washing in water, is then stained in 5 per cent. watery eosin for from 10 to 30 minutes, the specimens retaining much of the carbol-fuchsin requiring longer than those where most of the fuchsin has been washed out. Then, without washing, the specimen is transferred to ordinary methylene blue (alkalinity 1 in 1,000) ; it is examined from time to time under a low magnification to ascertain when the right differentiation has been obtained, and is then dehydrated and mounted. The time required for differentiation varies very considerably with different specimens (from a few seconds to a few minutes).

PHAGOCYTIC ACTION OF THE CALF'S FINELY GRANULAR OXYPHIL LEUOCYTES IN EARLY STAGES OF INFECTION WITH MAMMALIAN TUBERCLE BACILLI.

METHOD OF INOCULATION—SUBCUTANEOUS.

(*a.*)

BACILLI OF HIGH VIRULENCE.

DOSE FOR EACH ANIMAL—50 MG. OF BOVINE CULTURE.

Number of Calf.	Duration of Experiment.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
438	24 hours	Prescapular gland.	3	0	0	0
284	3 days	„	160	0	0	0
292	8 days	„ *	Abundant	49	3·2	Small
„	„	Lung	75	0	0	0
„	„	Liver	5	0	0	0
„	„	Spleen	14	0	0	0
288	14 days	Prescapular gland.*	Very abundant	211	13·0	Small
„	„	Lung	222	0	0	0
„	„	Liver	73	0	0	0
„	„	Spleen	194	0	0	0

SUMMARY (omitting specimens marked thus *).—In an area containing 8,000 leucocytes 746 bacilli were found, but none of these were within leucocytes.

(*b.*)

BACILLI OF LOWER VIRULENCE.

DOSE FOR EACH ANIMAL—50 MG. OF VIRUS H 26 K.M.

Number of Calf.	Duration of Experiment.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
873	3 days	Prescapular gland.	116	0	0	0
881	8 days	„	190	0	0	0
„	„	Lung*	0	0	0	0
„	„	Liver*	0	0	0	0
„	„	Spleen*	0	0	0	0
869	14 days	Prescapular gland.	234	7	·7	3·0
„	„	Lung	16	1	·1	6·2
„	„	Liver	2	0	0	0
„	„	Spleen	7	0	0	0

SUMMARY (omitting specimens marked thus *).—In an area containing 6,000 leucocytes 565 bacilli were found; but only 8 were within leucocytes, the percentage of leucocytes containing bacilli being ·1, and the percentage of bacilli within leucocytes 1·4.

PHAGOCYTIC ACTION OF THE CALF'S FINELY GRANULAR OXYPHIL LEUCOCYTES IN TERMINAL STAGES OF INFECTION WITH MAMMALIAN TUBERCLE BACILLI OF HIGH VIRULENCE.

METHOD OF INOCULATION—SUBCUTANEOUS.

DOSE FOR EACH ANIMAL—50 MG. OF CULTURE.

Number of Calf.	Virus.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
244	B IX.	19	Lung	892	101	6·7	11·3
"	"	"	Popliteal gland.	Abundant	242	18·5	Moderately high.
276	B XII.	24	Lung	Abundant	263	16·4	Moderately high.
"	"	"	Liver	280	4	·3	1·4
"	"	"	Popliteal gland.	Abundant (about 3,000).	168	8·7	Rather small.
250	B XIV.	30	Lung	1219	98	7·8	8
"	"	"	Liver	Abundant (about 2,000).	27	1·8	Small
"	"	"	Popliteal gland.	Abundant (about 3,000).	305	17	Moderately high.
522	EI.	36	Lung	Abundant	171	9·7	Rather small.
"	"	"	Liver	Abundant (about 2,000).	21	1·5	Very small.
"	"	"	Popliteal gland.	Abundant (about 3,000).	381	18·4	Rather high.
450	P XII.	41	Lung	Abundant	114	7·8	Rather small.
"	"	"	Liver	1290	28	1·9	2·2
"	"	"	Popliteal gland.	Abundant (about 2,000).	198	12·4	Moderately high.
1267	H 69. F.K.	56	Lung	Numerous (about 1,500).	28	2	Small.
"	"	"	Liver	391	0	0	0
"	"	"	Popliteal gland.	Abundant (about 2,000).	73	5·7	Small.
693	H 29. M.F.	63	Lung	Abundant (about 2,000).	119	8·3	Rather small.
"	"	"	Liver	Abundant (about 1,500).	14	1·1	Very small.
"	"	"	Thoracic gland.	Abundant (about 3,000).	90	6·6	Small.
440	E III.	72	Lung	2426	232	12·9	9·5
"	"	"	Liver	1343	0	0	0
"	"	"	Prepectoral gland.	Abundant (about 4,000).	302	14·7	Moderately high.
787	H 49. T.C.	81	Lung	Abundant (about 4,000).	271	20	Moderate.
"	"	"	Thoracic gland.	Abundant (about 4,000).	403	25·3	Moderately high.
448	P. XIV.	104	Lung	2251	382	14	16·9

SUMMARY.—In an area containing 26,000 leucocytes 4,035 bacilli were found within leucocytes, the percentage of leucocytes containing bacilli being 9·2.

PHAGOCYTIC ACTION OF THE CALF'S FINELY GRANULAR OXYPHIL LEUCOCYTES IN LATER STAGES OF INFECTION WITH MAMMALIAN TUBERCLE BACILLI OF RELATIVELY LOW VIRULENCE.

METHOD OF INOCULATION—SUBCUTANEOUS.

Number of Calf.	Virus.	Dose of Bacilli.	Duration of Experiment in days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
426	P. IV.	200 mg.	29	Lung	259	11	·7	4·2
”	”	”	”	Liver	6	0	0	0
”	”	”	”	Prescapular gland.	298	5	·4	1·7
185	H 9. C.T.	·75 cc.	40	Lung	4	0	0	0
”	”	”	”	Prescapular gland.	108	1	·1	1
165	H 18. T.T.	4,400,000	57	Lung	2	0	0	0
365	H 23. J.P.	100,000,000	61	Lung	2	0	0	0
”	”	”	”	Mesenteric gland.	91	0	0	0
498	E IV.	50 mg.	89	Lung	99	11	·5	11·1
273	H 16. J.H.	11,000,000	89	Lung	2	0	0	0
”	”	”	”	Thoracic gland.	6	0	0	0
312	P. IV.	50 mg.	90	Lung*	Abundant	113	7	Rather small.
406	E II.	10 mg.	91	Lung*	Abundant (about 4,000).	50	3·1	Small.
”	”	”	”	Mesenteric gland.*	Abundant	225	11·2	Moderate.

SUMMARY (omitting specimens marked thus *).—In an area containing 11,000 leucocytes 877 bacilli were found ; 28 of these were within leucocytes, the percentage of leucocytes containing bacilli being ·2, and the percentage of bacilli within leucocytes 3.2.

PHAGOCYTIC ACTION OF THE CALF'S FINELY GRANULAR OXYPHIL LEUCOCYTES UPON MAMMALIAN TUBERCLE BACILLI.

METHOD OF INOCULATION—INTRAVENOUS.

Number of Calf.	Virus.	Dose of Bacilli.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
529	H 17. Sp.B.	1,000,000,000	13	Lung*	Very abundant (about 5,000).	Small (Impossible to count).	Small	Very small.
”	”	”	”	Liver	1.500	0	0	0
”	”	”	”	Sternal gland.	43	0	0	0
831	H 25. A.T.	1,000,000,000	17	Lung	Abundant (about 2.000).	5	·5	Very small.
”	”	”	”	Liver	896	0	0	0
”	”	”	”	Iliac gland	300	0	0	0
859	H 25. A.T.	2,000,000,000	19	Lung	Abundant (about 3,000).	111	6·2	Very small.
”	”	”	”	Liver	369	0	0	0
89	H 2. Sp.B.	300,000	23	Lung	Abundant (about 2,000).	23	2	Very small.
”	”	”	”	Bronchial gland.	Abundant (about 3,000).	13	1	Very small.

PHAGOCYTIC ACTION OF THE CALF'S FINELY GRANULAR OXYPHIL LEUCOCYTES UPON MAMMALIAN TUBERCLE BACILLI—*continued.*

Number of Calf.	Virus.	Dose of Bacilli.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
749	**H 22. F.W.**	46 mg.	24	Lung	Abundant (about 2,000).	6	·6	Very small.
”	”	”	”	Liver	850	0	0	0
”	”	”	”	Mesenteric gland.	1,467	0	0	0
305	**H 8. S.C.**	730,000	26	Lung	Abundant (about 2,000).	0	0	0
”	”	”	”	Liver	606	0	0	0
”	”	”	”	Bronchial gland.	Abundant (about 2,000).	0	0	0
1373	**H 85. H.B.**	10 mg.	28	Lung	Numerous	58	3·3	Small.
”	”	”	”	Liver	1,600	0	0	0
”	”	”	”	Mediastinal gland.	Abundant (about 2,000).	0	0	0
399	**H 22. F.W.**	700,000,000	34	Lung	Abundant (about 3,000).	55	3·2	Very small.
”	”	”	”	Liver	Abundant (about 2,000).	5	·4	Very small.
”	”	”	”	Bronchial gland.	Abundant (about 2,000).	7	·7	Very small.
1391	**H 53. D.H.**	1 mg.	40	Lung	Very abundant	204	12	Rather small.
”	”	”	”	Liver*	0	0	0	0
”	”	”	”	Mediastinal gland.	Abundant (about 2,000).	90	5·3	Very small.
319	**H 12. H.N.**	100,000,000	57	Lung	Abundant (about 3,000).	118	6·5	Very small.
”	”	”	”	Liver	1,500	0	0	0
361	**H 8. S.C.**	41,500,000	76	Lung	6	0	0	0
”	”	”	”	Bronchial gland.	90	0	0	0

SUMMARY (omitting specimens marked thus *).—In an area containing 27,000 leucocytes 695 bacilli were found within leucocytes, the percentage of leucocytes containing bacilli being 1·5.

PHAGOCYTIC ACTION OF FINELY GRANULAR OXYPHIL LEUCOCYTES UPON MAMMALIAN TUBERCLE BACILLI IN CALVES PREVIOUSLY VACCINATED.

METHODS OF VACCINATION—VARIOUS (*see* pp. 27-33).

TEST DOSE FOR EACH VACCINATED ANIMAL—50 MG. OF B XXVI. (SUBCUTANEOUSLY INOCULATED).

Number of Calf.	Duration of Immunisation in Days.	*Duration of Experiment, in Days, after Test Inoculation.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
476	(Control)	D. 57	Lung	Abundant	173	15·2	Small.
”	”	”	Bronchial gland	Abundant	233	18·5	Small.
474	”	K. 92	Lung	135	4	·3	3
”	”	”	Bronchial gland	Numerous (about 2,000).	2	·2	Very small.
416	51	K. 89	Lung	63	0	0	0
”	”	”	Mediastinal gland	110	0	0	0
1027	63	D. 46	Lung	Numerous (about 2,000).	29	2·5	Very small.
”	”	”	Popliteal gland	8	0	0	0

PHAGOCYTIC ACTION OF FINELY GRANULAR OXYPHIL LEUCOCYTES UPON MAMMALIAN TUBERCLE BACILLI IN CALVES PREVIOUSLY VACCINATED—*continued.*

Number of Calf.	Duration of Immunisation in Days.	*Duration of Experiment, in Days, after Test Inoculation.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
1025	63	K. 91	Lung	9	0	0	0
„	„	„	Bronchial gland	6	0	0	0
1025	63	K. 92	Lung	Numerous (about 2,000).	0	0	0
„	„	„	Bronchial gland	44		0	0
1043	114	D. 53	Lung	27	0	0	0
„	„	„	Bronchial gland	24	0	0	0
1033	114	K. 90	Lung	18	0	0	0
„	„	„	Prescapular gland	6	0	0	0
1035	114	K. 91	Lung	26	0	0	0
„	„	„	Bronchial gland†	0	0	0	0
1039	114	K. 93	Lung	Numerous (about 2,000).	0	0	0
„	„	„	Prescapular gland	Numerous (about 2,000).	8	·8	Very small.
923	244	D. 36	Lung	408	8	·8	2
„	„	„	Mediastinal gland	204	2	·2	1

* D = Died. K = Killed.

SUMMARY (omitting specimen marked thus †).—In the tissues of the control animals 412 bacilli were present within leucocytes, in an area containing 4,000 leucocytes, the percentage of leucocytes containing bacilli being 8·5. In the tissues of the vaccinated animals 47 bacilli were present within leucocytes, in an area containing 17,000 leucocytes, the percentage of leucocytes containing bacilli being ·3.

PHAGOCYTIC ACTION OF THE SWINE'S FINELY GRANULAR OXYPHIL LEUCOCYTES IN EARLY STAGES OF INFECTION WITH MAMMALIAN TUBERCLE BACILLI OF HIGH VIRULENCE.

METHOD OF INOCULATION—SUBCUTANEOUS.

DOSE FOR EACH ANIMAL—20 MG. OF CULTURE OF VIRUS B IV.

Number of Pig.	Duration of Experiment.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
144	24 hours	Cervical gland.	179	6	·5	3·4
146	3 days	„	391	15	1·4	3·8
148	5 days	Cervical gland.	636	57	4·7	9·0
142	8 days	„	983	103	8·3	10·5
„	„	Lung	88	6	·6	6·8
150	14 days	Cervical* gland.	Abundant (about 2,000)	287	16·9	High
„	„	Lung	23	3	·3	13·0

SUMMARY (omitting the specimen marked thus *).—In an area containing 6,000 leucocytes 2,300 bacilli were found; 190 of these were within leucocytes, the percentage of leucocytes containing bacilli being 2·6, and the percentage of bacilli within leucocytes 8·3.

PHAGOCYTIC ACTION OF THE SWINE'S FINELY GRANULAR OXYPHIL LEUCOCYTES IN LATER STAGES OF INFECTION WITH MAMMALIAN TUBERCLE BACILLI.

Number of Pig.	Virus.	Mode of Infection.	Dose of Bacilli.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
18	B I.	Feeding	1,000,000	39	Lung	20	0	0	0
"	"	"	"	"	Mesenteric gland.	15	0	0	0
94	B IV.	Subcutaneous	1 mg.	61	Lung*	Abundant (about 3,000)	33	2·5	Small
"	"	"	"	"	Liver	50	0	0	0
"	"	"	"	"	Spleen	94	4	0·4	4·3
"	"	"	"	"	Mesenteric gland.*	Abundant (about 3,000)	47	3·9	Small
194	E III.	Subcutaneous	10 mg.	63	Lung	445	10	0·7	2·2
"	"	"	"	"	Liver	22	0	0	0
"	"	"	"	"	Spleen*	0	0	0	0
"	"	"	"	"	Submax. Lymph. gl.	43	0	0	0
49	H 50 P.H.	Subcutaneous	50 mg.	92	Lung	5	0	0	0
126	P IV.	Subcutaneous	1 mg.	114	Lung	23	0	0	0
20	B I.	Feeding	10,000,000	115	Lung	48	6	0·6	12·5
"	"	"	"	"	Submax. Lymph. gl.	20	2	0·2	10·0
80	B IV.	Feeding	10,000,000	131	Lung	43	1	0·1	2·3
92	B IV.	Feeding	10,000,000	137	Lung	1,100	18	1·4	1·6
"	"	"	"	"	Liver	49	0	0	0
"	"	"	"	"	Spleen	565	69	5·7	1·2
"	"	"	"	"	Tracheal gland.	198	7	0·7	3·5
186	E II.	Subcutaneous	50 mg.	174	Lung*	Abundant	Small	7·7	Small clumps.
"	"	"	"	"	Liver	5	0	0	0
"	"	"	"	"	Spleen	69	7	0·7	10
"	"	"	"	"	Bronchial gland.	187	9	0·7	4·6

SUMMARY (omitting specimens marked thus *).—In an area containing 19,000 leucocytes 3,001 bacilli have been found; 133 of these were within leucocytes the percentage of leucocytes containing bacilli being ·6, and the percentage of bacilli within leucocytes 4·4.

PHAGOCYTIC ACTION OF THE RABBIT'S FINELY GRANULAR OXYPHIL LEUCOCYTES IN EARLY STAGES OF INFECTION WITH MAMMALIAN TUBERCLE BACILLI OF RELATIVELY LOW VIRULENCE.

METHOD OF INOCULATION—INTRAVENOUS.

DOSE FOR EACH ANIMAL—1 MG. OF CULTURE OF VIRUS H 90 I.P.

Number of Animal.	Duration of Experiment.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
1567	24 hours	Lung	37	3	0·3	8·0
"	"	Liver	16	2	0·2	12·5
"	"	Spleen	6	2	0·2	33·0
1568	3 days	Lung	53	4	0·1	7·5
"	"	Liver	14	1	0·1	7·1
"	"	Spleen	21	5	0·3	23·8

PHAGOCYTIC ACTION OF THE RABBIT'S FINELY GRANULAR OXYPHIL LEUCOCYTES IN EARLY STAGES OF INFECTION WITH MAMMALIAN TUBERCLE BACILLI OF RELATIVELY LOW VIRULENCE—*continued.*

Number of Animal.	Duration of Experiment.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
1570	5 days	Lung	138	8	0·7	5·8
"	"	Liver	13	0	0	0
"	"	Spleen	18	4	0·2	22·0
1571	8 days	Lung	454	25	2·0	5·5
,	"	Liver	116	9	0·5	8
"	"	Spleen	7	0	0	0
1569	14 days	Lung	141	6	0·6	4·2
"	"	Liver	133	0	0	0
"	"	Spleen	23	0	0	

SUMMARY.—In an area containing 15,000 leucocytes 1,102 bacilli were found; 60 of these were within leucocytes the percentage of leucocytes containing bacilli being ·3, and the percentage of bacilli within leucocytes 5·4.

PHAGOCYTIC ACTION OF THE RABBIT'S FINELY GRANULAR OXYPHIL LEUCOCYTES IN EARLY STAGES OF INFECTION WITH MAMMALIAN TUBERCLE BACILLI OF HIGH VIRULENCE.

I.

METHOD OF INOCULATION—INTRAVENOUS.

DOSE FOR EACH ANIMAL—1 MG. OF CULTURE OF VIRUS B III.

Number of Animal.	Duration of Experiment.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
29	24 hours	Lung	90	9	0·5	10·0
"	"	Liver	64	8	0·6	8·0
"	"	Spleen*	0	0	0	0
33	3 days	Lung	85	5	0·4	5·8
"	"	Liver	144	12	0·8	8·3
"	"	Spleen	26	2	0·2	7·7
40	5 days	Lung	253	8	0·8	3·1
"	"	Liver	561	5	0·4	0·9
"	"	Spleen	100	6	0·6	6·0
31	10 days	Lung*	Abundant	15	1·0	Small.
"	"	Liver	1,498	16	1·5	1·1
"	"	Spleen	421	15	1·1	3·5
30	15 days	Lung*	Very abundant	74	5·9	Small.
"	"	Liver*	Abundant	15	1·3	Small.
"	"	Spleen	756	19	1·7	2·5

II.

METHOD OF INOCULATION—INTRAVENOUS.

DOSE FOR EACH ANIMAL—1 MG. OF CULTURE OF VIRUS H 7 C.M.

42	24 hours	Lung	230	32	2·4	13·9
"	"	Liver	45	3	0·3	6·6
"	"	Spleen*	0	0	0	0

PHAGOCYTIC ACTION OF THE RABBIT'S FINELY GRANULAR OXYPHIL LEUCOCYTES IN EARLY STAGES OF INFECTION WITH MAMMALIAN TUBERCLE BACILLI OF HIGH VIRULENCE—*continued.*

Number of Animal.	Duration of Experiment.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
40	3 days	Lung	125	10	0·8	8·0
,,	,,	Liver	189	11	1·0	5·8
,,	,,	Spleen	19	3	0·2	15·8
50	5 days	Lung	184	10	0·8	5·5
,,	,,	Liver	103	5	0·5	4·8
,,	,,	Spleen	22	2	0·2	9·0
49	10 days	Lung*	Abundant	46	3·0	Small.
,,	,,	Liver	1,096	4	0·4	0·4
,,	,,	Spleen	461	15	1·3	3·3
48	15 days	Lung*	Very abundant	75	5·9	Small.
,,	,,	Liver*	Abundant	13	1·0	Small.
,,	,,	Spleen	559	15	1·3	2·7

SUMMARY of I and II (omitting specimens marked thus *).—In an area containing 22,000 leucocytes 7,031 bacilli were found; 215 of these were within leucocytes, the percentage of leucocytes containing bacilli being ·8, and the percentage of bacilli within leucocytes 3·1.

PHAGOCYTIC ACTION OF THE RABBIT'S FINELY GRANULAR OXYPHIL LEUCOCYTES UPON MAMMALIAN TUBERCLE BACILLI.

LATER STAGES AFTER INTRAVENOUS INOCULATION.

Number of Animal.	Virus.	Dose of Bacilli.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes
36	B III.	1 mg.	18	Lung*	Abundant	Fairly numerous (uncountable).	Moderate	Rather small.
,,	,,	,,	,,	Liver*	Abundant	Fairly numerous (uncountable).	Moderate	Rather small.
,,	,,	,,	,,	Spleen	4,104	188	13	4·5
1215	H 78. O.D.	mg.	18	Lung	87	0	0	0
,,	,,	,,	,,	Liver	79	0	0	0
,,	,,	,,	,,	Spleen	80	0	0	0
37	B III.	1 mg.	23	Lung*	Very abundant.	Fairly numerous (uncountable.)	Moderate	Moderate.
,,	,,	,,	,,	Liver	Numerous	35	2·6	Small.
,,	,,	,,	,,	Spleen	Numerous	5	·4	Very small.
1246	H 77. R.L.	·1 mg.	23	Lung	Numerous	91	5·3	Rather small.
,,	,,	,,	,,	Liver	Numerous	5	·5	Very small.
,,	,,	,,	,,	Spleen	Numerous	0	0	0

PHAGOCYTIC ACTION OF THE RABBIT'S FINELY GRANULAR OXYPHIL LEUCOCYTES UPON MAMMALIAN TUBERCLE BACILLI—*continued.*

Number of Animal.	Virus.	Dose of Bacilli.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
43	H 7. C.M.	1 mg.	24	Lung*	Abundant	Fairly numerous (uncountable).	Moderate	Rather small.
"	"	"	"	Liver	Numerous	13	1·1	Very small.
"	"	"	"	Spleen	Numerous	2	·2	Very small.
1342	H 87. W.D.	10 mg.	26	Lung	273	7	·7	2·6
"	"	"	"	Liver	55	0	0	0
"	"	"	"	Spleen	19	0	0	0
1212	H 78. O.D.	1 mg.	28	Lung	380	4	·4	1
"	"	"	"	Liver	34	0	0	0
"	"	"	"	Spleen	6	0	0	0

SUMMARY (omitting specimens marked thus *).—In an area containing 17,000 leucocytes 350 bacilli have been found within leucocytes, the percentage of leucocytes containing bacilli being 1·4.

PHAGOCYTIC ACTION OF THE GOAT'S FINELY GRANULAR OXYPHIL LEUCOCYTES IN TISSUES INFECTED WITH MAMMALIAN TUBERCLE BACILLI.

Number of Goat.	Virus.	Mode of Infection.	Dose of Bacilli.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
7	H 32 Y.W.	Subcutaneous	155,000,000	27	Lung	248	3	·3	1·2
"	"	"	"	"	Liver	12	0	0	0
"	"	"	"	"	Thoracic gland.	101	0	0	0
1	Three human viruses.	Subcutaneous	620,600,000 in the last inoculation.	34 (after the last inoculation).	Lung†	Abundant (about 4,000).	173	9·6	Small.
19	Two human viruses.	Subcutaneous	310,330,000 in the last inoculation	35 (after the last inoculation).	Lung†	Abundant (about 2,500).	86	5·7	Small.
"	"	"	"	"	Thoracic gland.	405	0	0	0
2	B V.	Subcutaneous	4,500,000	54	Lung	172	0	0	0
"	"	"	"	"	Prescapular gland.	144	0	0	0
5	H 14 F. S.	Subcutaneous	4,500,000	75	Lung†	174	42	3·0	24·1*
"	"	"	"	"	Thoracic gland.	11	0	0	0
39	H 17 Sp. B.	Subcutaneous	102,000,000	92	Lung	32	0	0	0
"	"	"	"	"	Bronchial gland.	12	0	0	0
35	H 31 L.F.	Feeding	10 mg.	106	Lung	61	0	0	0
"	"	"	"	"	Thoracic gland.	16	0	0	0
20	B IV.	Feeding	10,150,000	131	Lung†	Abundant (about 2,000).	66	3·5	Small.
"	"	"	"	"	Cervical gland†.	Abundant (about 3,000).	75	4·3	Small.

* All the 42 bacilli found within leucocytes were contained in a bronchus.

SUMMARY (omitting specimens marked thus †).—In an area containing 11,000 leucocytes 1,214 bacilli have been found; only 3 of these were within leucocytes.

A COMPARISON OF FINELY GRANULAR OXYPHIL LEUCOCYTES IN THE BOVINE, PIG, AND RABBIT.

Taking only the specimens where bacilli were present, were countable and were mammalian in type, the following results are obtainable from the foregoing tables :—

Species of Animal.	Leucocytes counted.	No. of Bacilli present in this area.	No. of Bacilli in Leucocytes.	Percentage of Bacilli in Leucocytes.
Bovines	25,000	2,188	36	1·6
Swine	25,000	5,301	323	6·1
Rabbits	37,000	8,133	275	3·4

C.—THE RELATION OF SMALL LYMPHOCYTES TO MAMMALIAN TUBERCLE BACILLI.

(1.) At the Site of Inoculation.

Calf 438.—Killed 24 hours after inoculation with 44 mg. Small lymphocytes are rare. The bacilli are surrounded with an enormous number of multinuclear leucocytes.

Calves 284 and 873.—Killed three days after inoculation with 50 mg. Compared with the 24 hours' specimen, small lymphocytes are commoner, both amongst the large mass of bacilli and also in the peripheral parts of the lesion where bacilli are not very numerous. The lymphocytes are irregularly distributed, do not occur in groups, and are very much fewer than the leucocytes.

Calves 292 and 881.—Killed eight days after inoculation with 50 mg. Compared with the three days' specimens, there is a general increase in the number of small lymphocytes. As before, these cells are irregularly scattered and do not form groups ; they appear to be avoiding the areas which contain dense masses of bacilli and are breaking down.

Calves 288 and 869.—Killed 14 days after inoculation with 50 mg. There is again a marked increase in the number of small lymphocytes. They often occur in groups, especially amongst loose connective tissue. Lymphocytes are more numerous in Calf 869 (inoculated with bacilli of low virulence) than in Calf 288 (inoculated with bacilli of high virulence).

Calf 769.—Died, from intercurrent disease, 17 days after inoculation with 50 mg. of a slightly virulent virus. Small lymphocytes are very numerous ; they occur in groups and are also loosely distributed throughout the tissue. Multinuclear leucocytes are also numerous.

Calf 767.—Killed 25 days after inoculation with 50 mg. of a culture slightly virulent for the bovine. Amongst the fibro-caseous walls of the lesion there is a considerable sprinkling of small lymphocytes, but these cells are much less numerous than plasma cells.

Calf 185.—Killed 40 days after inoculation with ·75 cc. of an old culture of a virus of low virulence for the bovine. External to the caseous areas of the lesion small lymphocytes are very abundant and are greatly in excess of the leucocytes.

Calf 105.—Killed, when moribund, 47 days after inoculation with tissue containing 48,000 bacilli of high bovine virulence. Small lymphocytes are fairly abundant at the periphery of caseous areas, where leucocytes are also abundant. The lymphocytes do not form such large collections as are often found in lesions of a more chronic type.

Calf 510.—Killed 96 days after inoculation with 50 mg. of culture slightly virulent for the bovine. The lesion is separated from the subcutaneous muscle by a dense fibrous wall, in the interstices of which groups of small lymphocytes are found. Between the fibrous wall and the caseous interior of the lesion there is a cellular zone amongst which small lymphocytes are very plentiful.

Calf 267.—Killed 111 days after inoculation with a very large dose of dead culture belonging to a virus of low virulence for the bovine. In the lesion, which is only slightly caseous, there are large groups of small lymphocytes, and these cells are also scattered abundantly throughout the tissue.

Heifer 81.—Killed 132 days after inoculation with an emulsion of human tissue containing bacilli of high virulence for the bovine. Near the caseous areas small lymphocytes are scattered irregularly, and at the periphery of the lesion these cells are often collected into groups.

(2.) In Disseminated Lesions during the First Fortnight.

TWENTY-FOUR HOURS.

Two rabbits were inoculated intravenously with highly virulent bacilli (dose for each, 1 mg.) and were killed 24 hours afterwards. In the lungs of both animals small lymphocytes, though much less numerous than leucocytes, are in excess of the normal and are generally distributed ; lymphocytes are more numerous in the lungs of one animal than in the other. In the livers of both animals the bacilli are in close contact with oxyphil leucocytes ; near to these cells, but not in such close proximity to the bacilli, there is a somewhat smaller number of small lymphocytes.

THREE DAYS.

Two rabbits were killed three days after intravenous inoculation with the same dose of the same material as the last. In both animals the lungs show an increase in the number of small lymphocytes, these cells being quite as numerous as the leucocytes both within the lesions and outside. In the livers both lymphocytes and leucocytes are present in the lesions ; in the tissues as a whole these two types of cells are about equally represented, but in some of these lesions lymphocytes are in excess.

FIVE DAYS.

Two rabbits were killed five days after intravenous inoculation with the same dose of the same material as the last. There is again an increase in the number of small lymphocytes, both in the lungs and liver, these cells now being decidedly more numerous than the leucocytes.

EIGHT DAYS.

Calf 292.—Killed eight days after subcutaneous inoculation with 30 mg. of bovine bacilli. In the lungs, where there are many small deposits of bacilli which have produced minute early lesions, oxyphil leucocytes are in excess of the normal both in the lesions and within blood-vessels. With the leucocytes small lymphocytes are associated, but are less numerous. In the liver, where bacilli are rare, there is a slight excess of both leucocytes and lymphocytes, particularly in association with the lesions; but the proportion of lymphocytes to leucocytes is greater than in the lungs.

Pig 142.—Killed eight days after subcutaneous inoculation with 20 mg. of bovine bacilli. In the lungs bacilli are fairly numerous and are irregularly distributed throughout the tissue; they have given rise to many early tubercles. Leucocytes are very abundant, and small lymphocytes are always associated with them. Within the lesions the lymphocytes are relatively few, compared with the leucocytes; in the rest of the tissue they are almost as numerous as the leucocytes. In the liver bacilli are rare, and there are only a few early tubercles. Lymphocytes are as numerous as leucocytes in this organ, both within the lesions and in the rest of the tissue.

TEN DAYS.

Two rabbits were killed 10 days after intravenous inoculation with the same dose of the same material as the rabbits mentioned above. In the lungs of both animals small lymphocytes are very numerous; there is a great increase in the number of oxyphil leucocytes, as compared with the five days' specimens. In the livers, small lymphocytes are greatly in excess of leucocytes at the periphery of the lesions and in the tissue apart from the lesions; within the interior of the lesions there are often several leucocytes but no lymphocytes. In the kidneys both lymphocytes and leucocytes are numerous in the lesions.

THIRTEEN DAYS.

Calf 529.—Died thirteen days after intravenous inoculation with a tissue emulsion containing about 1,000 million bacilli. In the lungs, where bacilli are very abundant, small lymphocytes are numerous in the tissue generally, and are also collected at the periphery of the lesions. In the liver small lymphocytes are much less numerous. This organ shows diffuse necrotic areas suggestive of a toxic infection and also smaller lesions more resembling tubercular foci.

FOURTEEN DAYS.

Calf 288.—Killed 14 days after subcutaneous inoculation with 50 mg. of bovine bacilli. In the lungs both lesions and bacilli are much more numerous than in Calf 292 (eight days), but the relations and distribution of the lymphocytes is the same, these cells being less numerous than the leucocytes. In the liver, where there are many small tubercles, small lymphocytes are relatively more frequent, and in some of the lesions are about twice as numerous as the leucocytes; the lymphocytes are also freely distributed in the circulation of this organ. In lesions found in the kidneys lymphocytes are also fairly numerous.

Calf 869.—Killed 14 days after subcutaneous inoculation with 50 mg. of bacilli slightly virulent for the bovine. The lungs contain a few small tubercles, in which bacilli are scanty. Lymphocytes are present in the lesions, but are less numerous than leucocytes.

Pig 150.—Killed 14 days after subcutaneous inoculation with 20 mg. of bovine bacilli. In the lungs the lesions are rather more advanced than in Pig 142 (eight days), but bacilli do not appear to be more numerous in individual lesions. Both lymphocytes and leucocytes are more numerous than in the eight days' specimen, but there is the same relation between them. In the liver, where there are a few small lesions and bacilli are scanty, lymphocytes are moderately numerous in the lesions and are rather in excess of the leucocytes.

(3.) Later Lesions—Acute.

In rabbits infected by intravenous inoculation of virulent bacilli no great change is observed in the small lymphocytes from the tenth day until the disease terminates fatally (between the third and fourth week). In two rabbits killed on the 15th day the lungs show a great increase in the number of oxyphil leucocytes, but the small lymphocytes are still very numerous except in the interior of the lesions, which are becoming necrotic. In the livers the lymphocytes are more numerous than the leucocytes. In the kidneys, where there are only slight lesions, lymphocytes are not particularly numerous. In rabbits similarly inoculated which died at later periods (18th to 24th day) the lymphocytes in the lungs and liver show no change as compared with the 15 days' specimens; the kidneys show rather more advanced lesions, which contain more lymphocytes.

In calves fatally infected by subcutaneous inoculation of large doses, the number of small lymphocytes present in the lesions at the time of death varies considerably in different specimens. In the lungs they are usually abundant, but owing to the uniformity of their distribution they do not make a prominent feature in the general structure of the tissue. Though generally distributed, they tend to be more numerous at the margins of the lesions than elsewhere, but in these situations they are collected in a loose and irregular fashion which is in contrast to the more copious and denser aggregation of these cells found at the periphery of lesions of a more chronic type. In the livers also the small lymphocytes vary considerably. In some specimens, generally those in which the oxyphil leucocytes are particularly numerous, the lymphocytes are relatively scanty; and in others the lymphocytes outnumber the leucocytes. The lymphocytes tend to aggregate round the margins of the tubercles, particularly those which are centrally caseous, and along tracts of interlobular connective tissue; but these aggregations are less dense than those found in chronic lesions. In the kidneys small lymphocytes occur abundantly at the periphery of caseous lesions and within the areas of early infiltration; they generally greatly outnumber the leucocytes.

In calves fatally infected by intravenous inoculation no differences of importance have been observed as regards the small lymphocytes. As in Calf 529, the lungs of animals dying at later periods generally contain large numbers of these cells. In the livers the condition is more variable. Where there is diffuse necrotic change suggestive of intoxication, the lymphocytes are not abundant; they are more numerous where this condition is absent and where the ordinary tissue changes associated with tuberculosis are in progress.

(4.) Later Lesions—Less Acute and Chronic.

In lesions of intermediate severity there is a tendency for the small lymphocytes to increase in number at the periphery of the lesions and to find their way into the interior. For example, in the lungs of Calf 340, killed 92 days after subcutaneous inoculation with 10 mg. of bovine bacilli, there are many caseous areas which appear to be coalescing, though they show a tendency to fibroid demarcation at their periphery; small lymphocytes are abundant throughout the tissue even in the areas of early caseation; but they are not closely packed together and do not form a definite zone at the periphery of the lesions. The lungs of Calf 312, killed 90 days after subcutaneous inoculation with 50 mg. of a porcine virus of relatively low virulence, also illustrate lesions of the intermediate type. In this case the infiltration of small lymphocytes at the periphery of the caseous lesions is denser than in Calf 340, and many of the smaller and non-caseous tubercles are crowded with these cells. The lungs of Calf 905, killed 90 days after subcutaneous inoculation with 50 mg. of a virus possessing an intermediate degree of virulence, also show slowly progressive semi-confluent lesions; in this specimen small lymphocytes are very abundant and are often collected into large groups.

But the number of small lymphocytes present in the less severe infections is very variable, and it cannot be laid down as a general rule that the increase of these cells is proportionate to the diminution in the severity of the infection. In the tissues of many rabbits showing slowly progressive disease the small lymphocytes, particularly in the lungs, are relatively inconspicuous, whereas oxyphil leucocytes are abundant.

In the chronic and definitely retrogressive lesions there is again a great deal of variation in the number of small lymphocytes present. In lesions which consist merely of a caseo-calcareous centre surrounded by compact, non-vascularised, fibrous tissue, lymphocytes are rare; where the fibrous zone is less dense, small islands of lymphocytes may be found in the gaps; and where there is a cellular zone internal to the fibrous periphery, these cells are generally fairly plentiful. Then caseating and obviously retrogressive foci are often met with which show little or no evidence of a fibrous periphery. When the development of these lesions is arrested at an early stage, just at the commencement of caseation, small lymphocytes make their way into the interior of the lesion; they may completely fill it up, rendering it almost indistinguishable from a nodule of normal lymphoid tissue, or they may permeate it less completely, leaving small gaps where giant cells are generally seen. And frequently the central focus becomes completely caseous or calcareous; surrounding this there is a broad zone of cells consisting almost entirely of small lymphocytes; interspersed amongst these there may be a few oxyphil leucocytes, but there is very little evidence of a fibrous or endothelial stroma.

D.—THE RELATION OF ENDOTHELIAL CELLS TO MAMMALIAN TUBERCLE BACILLI.

(1.) Swelling.

(a) IN CELLS RETAINING THEIR CONNECTION WITH AN ENDOTHELIAL LINING.

Except in lesions which have healed or are at least definitely quiescent, swelling of the endothelial cells is constantly found in tissues infected with the tubercle bacillus.

In the local lesions produced in calves by subcutaneous inoculation this swelling is generally very conspicuous, particularly in the early periods of tissue change. In a specimen removed 24 hours after inoculation (Calf 438), the predominant feature is the invasion of leucocytes, and the reaction of the fixed tissue elements has hardly commenced, but the endothelium of the capillaries is slightly swollen. In three days' local lesions (Calves 284 and 873) the swelling of the endothelium is very conspicuous and is found in all parts of the tissue. In eight days' specimens (Calves 292 and 881) many of the endothelial cells are beginning to be affected by degenerative processes, and though numerous examples of swollen endothelial cells are met with, the general impression conveyed by the specimens is that this tendency is less marked than in the three days' specimens. In 14 days' specimens (Calves 288 and 869) the degenerative processes are further advanced, but apart from these areas the swelling of the endothelial cells is again very conspicuous and recalls the condition found in the three days' specimens. Examples of later local lesions need not be quoted in detail. They all serve to illustrate the general fact that except in situations where the disease is quiescent or where degenerative changes predominate, the endothelial cells are generally swollen.

The same tendency of the endothelial cells to swell up is also observed in the other tissues of the body. It is well seen in early infections of the liver. In a series of rabbits killed at short intervals after intravenous inoculation with 1 mg. of virulent bacilli this swelling is conspicuous as early as the first day and is found in all the animals killed at later periods, the number of swollen endothelial cells progressively increasing. The same change is observed in the livers of rabbits intravenously inoculated with dead bacilli, *e.g.* in Rabbits 1283 and 1284, killed 29 days after doses, respectively, of 50 and 10 mg. of bovine bacilli, and in Rabbit 1195, killed 21 days after a dose of 50 mg. of human bacilli belonging to a virus of relatively low virulence. The livers of two pigs (Pigs 142 and 150), killed eight and 14 days after subcutaneous inoculation with 20 mg. of virulent bovine bacilli, also show a notable swelling of the endothelial cells. The swelling up of the endothelial cells is also one of the earliest changes observed in lesions of the lungs, and the same feature is found in early lesions involving the lymph sinuses of lymphatic glands. In later lesions, in all organs of the body, the character of the endothelial cells varies according to the nature of the lesions with which they are associated, the general condition being the same as that observed in the local lesions, *i.e.* when the lesions are neither quiescent nor undergoing rapid degenerative change, swelling of the endothelial cells in their neighbourhood is generally conspicuous

(b) IN CELLS DETACHED FROM ANY CONTINUOUS ENDOTHELIAL LINING.

(α) *Cells carried into the blood or lymph.*—When an endothelial cell swells up, it very frequently detaches itself from the lining membrane of which it is a constituent, and then becomes a free cell in the fluid contained within the endothelial lining. This detachment of endothelial cells is an important feature of tissues infected with tubercle bacilli; it occurs within blood-vessels and capillaries, within lymph channels and sinuses, and within larger spaces lined by endothelium, such as the pleural and peritoneal cavities. This phenomenon is not confined to the earlier stages of infection, but may be observed wherever the infective process is active and progressive. These detached cells possess all the characteristics of cells sometimes described as "macrophages."

(β) *Detached Cells found amongst other Cellular Elements.*—When an endothelial cell swells up and severs its normal connection with a lining membrane, instead of passing into the blood or lymph it may, owing to various reasons, such as the break in continuity of the vessel wall or the occlusion of the vessel or capillary, find itself lying amongst other cellular elements which are not in contact with either blood or lymph channels. Changes of this nature are constantly going on in the process of tubercle formation, and when the process has not advanced too far the endothelial character of these swollen cells can be verified by observing that they belong to the wall of a partially obliterated capillary channel. Cells in this condition are identical in character with what are often termed "epithelioid" cells.

(2.) Diffuse Staining of Nucleus.

A noticeable feature about endothelial cells present within or near infected sites is that the nuclei of some of them do not stain sharply. In these cases the stain is generally rather deeper than that taken up by a normal nucleus, but in addition to this feature there is a want of definition, giving the impression, at first sight, that the excess of stain has not been properly washed out. But closer examination shows that the staining is not at fault; the blurred appearance is confined to sites where there is evidence of pathological change, and the want of definition is apparently due to a diffusion of slightly basophil material throughout the nucleus and into the surrounding protoplasm. This condition is most conspicuous in animals killed a short time after inoculation, *e.g.* in the tissues of rabbits intravenously inoculated, in the local lesions of calves subcutaneously inoculated, or in tissues illustrating early dissemination in the calf or pig after subcutaneous inoculation. The diffuse nuclear staining is observable both in single endothelial cells which are not associated with other tissue changes and in endothelial cells which are present within, or at the periphery of, early tubercles. Accompanying this change there is a dissolution of continuity in the protoplasmic outline of the endothelial cell, and a tendency for the protoplasm of these cells to fuse with the protoplasm of the parenchymatous or other cells with which they happen to be in contact. This tendency to protoplasmic fusion is well shown in specimens of livers showing early lesions, and is the explanation of the difficulty constantly met with in attempting to follow up the parts played respectively by the endothelial cells and the parenchymatous cells of the liver in the formation of tubercles. In the present connection the special importance of this feature lies in the fact that when the nucleus of the endothelial cell loses its integrity as a sharply defined body, the protoplasm with which the diffuse nuclear material is in immediate contact is not merely endothelial cell protoplasm but consists of heterogeneous elements. It must also be added that concurrently with this tendency to a partial loss of nuclear individuality another process is at work, a tendency for the nuclei to divide by direct fission, and thus to regain a clearly defined contour.

(3.) Increase of Nuclei by Direct Division.

When the nucleus of an endothelial cell finds itself in a focus which is developing into a tubercle, the commonest thing which happens is that the nucleus loses its regularity of outline. It may show a shallow or a deep indentation on one side or on opposite sides. There may be a globular bulging from some part of its periphery; the nucleus may be narrow and elongated, or it may appear irregularly compressed; in short, it may assume any conceivable irregularity of form. These variations all indicate that the factors determining the normal morphology of the nucleus have got out of control. In the neighbourhood of such nuclei as these there are generally found others in which one or more dividing lines may be seen. These lines may run along the short axis of the nucleus or along the long axis, or they may run more or less obliquely; and two or more such lines may be found in the same nucleus. It is frequently possible to observe near at hand the next stage in the process, the formation of a small group of nuclei, each with a well defined contour. A further stage in the process which is frequently observed is that these groups of nuclei continue, for a time, to multiply; they then form large groups which, taken in conjunction with their protoplasmic environment, are termed "giant cells." But it must not be taken for granted that giant cells in general or even the giant cells specially referred to here all develop from endothelial cells and nothing but endothelial cells. It was pointed out in the last section that when the nucleus of an endothelial cell begins to lose its individuality, its protoplasm also becomes merged with the protoplasm of other cells contiguous to it. And when nuclear multiplication has taken place the resulting multinucleate cell, or rather the collection of nuclei in a protoplasmic matrix, is the joint product of the material previously existing in this area. The multinucleate cell mass develops concurrently with the loss of individuality in the pre-existing material, and develops out of this material as a whole. In the liver of the rabbit, for example, it is often found that there is first a stage where there is a blending of endothelial and parenchymatous cells; this gradually passes into a phase where there is simply a collection of nuclei in a homogeneous protoplasmic matrix. Owing to the loss of individuality both of protoplasm and of nuclei, it is impossible to regard the resultant growth as either merely endothelial or merely parenchymatous. Hence, to return to the part played by endothelial cells in tubercle formation, it is characteristic of these cells to divide amitotically, but thereupon they cease to be endothelial cells since there is no concomitant division of endothelial protoplasm.

This tendency for the nucleus of the endothelial cell to multiply by direct division is very common and may be found in the majority of tubercles. It has an important bearing upon the question of the formation of giant cells. When seen under a low power the giant cell stands out conspicuously from the surrounding confusion of nuclei, protoplasm, and caseation; and hence one is apt to regard it as a specialised structure. But on closer examination it is seen to be no more than the culminating point of this tendency to nuclear multiplication by direct division. The first phase of this process is the irregular bulging or indenting of a nucleus; at this stage the process may stop and degeneration may set in. But if the vitality of the nucleus is not yet completely exhausted, the process continues and we have division by amitosis; at this stage we have a small group of nuclei, more or less imperfectly formed and generally stuck together. Here again the process may stop, and be followed by degeneration. Or this stage may be followed by further division of the nuclei, some of the new nuclei becoming clearly separable from the original group; at this point, which also may be the final stage prior to degeneration, we find nuclei scattered about loosely in a vaguely defined mass of protoplasm; these nuclei are often round and about the size of small lymphocytes, but may be distinguished from the latter by their lighter staining properties. And, lastly, the nuclei may grow into a compact group occupying either the centre or the periphery of the protoplasmic mass; this final stage is termed giant-cell formation. The giant cell is generally a hybrid product, however, and is not of purely endothelial origin.

(4.) Proliferation.

At an early stage after infection regular mitotic figures may be found in endothelial cells which have not become separated from their normal surroundings.

In the local lesions of Calves 284 and 873 (killed three days after subcutaneous inoculation) numerous mitoses are found in the capillary endothelium (as many as six in travelling in a straight line across a small section). In the lesion of Calf 292 (eight days) no mitoses have been found in the specimen examined, but many of the endothelial cells appear to be breaking down. In the lesion of Calf 881 (eight days) several mitotic figures are present, but the cells do not stain as sharply as in the three days' specimens, and many of them appear to be breaking down. In the lesion of Calf 288 (14 days) there are many mitoses of the endothelial cells, but degenerative changes are also noticeable; and in the lesion of Calf 869 (14 days), which shows more tendency to conservative change, a few mitoses of endothelial cells have been found.

In a series of rabbits killed at short intervals after intravenous inoculation with 1 mg. of virulent bacilli the livers have been examined with a view to observing mitoses in endothelial cells. In the 24 hours' specimens a few nuclei have been found which stain deeply and appear to be in the stage preparatory to mitosis, but no clearly defined mitotic figures have been observed. In the three and five days' specimens there are a few mitoses; some are also found at the margins of the tubercles in the 10 days' specimens.

These examples show that there is a tendency, in the early period after infection, for endothelial cells to proliferate by the normal method of nuclear division. When definite lesions are in process of development a general distinction may be drawn between the endothelial cells (1) within, (2) at the periphery of, and (3) external to the periphery of the lesions.

(1) The cells which become definitely included within the lesions very frequently divide amitotically, but do not exhibit mitotic figures; and the products of these amitotic divisions show no clearly defined protoplasmic outline; though generally forming conspicuous elements in the lesions, they must be regarded as having lost their individuality as endothelial cells; there is no indication that they are capable of progressive development, but, on the contrary, there is strong evidence that they gradually

give way to caseous change. Amongst the endothelial cells included within caseous areas, those which constitute the boundary of blood capillaries are of particular importance. These cells may show a swelling of the nucleus or other irregularities, but they find it impossible to proliferate in the midst of caseous material, and their usual fate is to break down.

(2.) At the periphery of the lesions two processes can generally be found at work. (*a*) There is the endothelial cell which tends to divide amitotically and, by contributing to the progressive changes described above under the head of direct division, plays a part in the production of multinucleate cell masses and giant cells. In short, the endothelial cell at the periphery of an expanding lesion plays the same part as the endothelial cells found in the centres of minute, newly formed tubercles. (*b*) Occasionally a cell is found in mitotic division; and, when no mitotic figures can be found, there is often well marked evidence of endothelial proliferation. A characteristic feature is the tendency to the formation of new capillary channels. These may be found, partly filled with red blood-corpuscles, running in all directions and ending abruptly. They often pass directly inwards and reach as far as the caseous material. It is also common to find these cells attached end to end and running in single lines. Isolated cells, identical in character with cells forming new capillary channels, are also frequent; many of these are probably derived from endothelium, though their morphological appearance does not definitely prove their origin, since young connective tissue corpuscles may assume similar characters.

(3.) External to the periphery of the lesions the condition of the endothelial cells depends on the type of infection. If the lesion is of a definitely retrogressive type and is surrounded by a firm fibrous layer, the endothelial cells in the outlying regions may show little or no change; and in acute lesions accompanied by rapid tissue destruction, the endothelial cells outside the foci are generally swollen but show no sign of proliferation. But proliferation of the outlying endothelial cells is generally found in the sub-acute or slowly progressive infections, and this activity is accompanied by a formation of new vascular channels. The vascularised growths found in the bovine projecting from the surface of the lungs and liver are good examples of this condition.

(5.) Relations of Endothelial Cells to Bacilli.

(*a*) NORMAL CELLS.

When bacilli are carried into the blood or lymph stream, they endeavour to escape from these media, and the majority of them succeed in doing so within a short space of time. This is shown, in the case of the blood, by the rapid escape of the great majority of the bacilli into the tissues after intravenous inoculation; and, in the case of the lymph, by the rapid disappearance of free bacilli from lymph channels and sinuses in regions which drain the site of a subcutaneous inoculation. In the course of dissemination bacilli must therefore be constantly coming into contact with normal endothelial cells, and histological sections sometimes happen to pass through endothelium showing bacilli in this condition. The endothelial cells readily ingest the bacilli: but it is rare to find such cells retaining their normal shape and relationship. As I have examined a very large number of specimens abundantly infected with bacilli, I conclude from the rarity of this condition that the endothelial cell is unable, or rarely able, to simply digest the ingested bacilli and then resume its normal characters. As to the possibility of a bacillus making its escape by passing between contiguous endothelial cells, I can only say that it is difficult to demonstrate this process but it seems possible that a direct escape in this way through an intact endothelium may occur.

(*b*) SWOLLEN CELLS.

(*α*) *Cells forming part of an Endothelial Lining.*—Only a relatively small number of the swollen cells found in their normal position actually contain bacilli. This condition of the cells in an infected tissue is evidently due to a diffusible stimulus, and not necessarily to the presence of bacilli.

(*β*) *Cells lying free in Blood or Lymph Channels.*—A considerable number of these detached endothelial cells contain bacilli. Probably many of them have received the bacilli before they become swollen up and detached from the endothelial wall, but it is also possible that some of them have picked up bacilli after they have become free cells.

(*γ*) *Cells lying free in the Tissues apart from Blood or Lymph Channels.*—Endothelial cells in these situations are a favourite site for the tubercle bacillus; a single cell may frequently contain several bacilli, and sometimes the bacilli are so numerous that it is evident that this position has proved favourable to their multiplication. As the bacilli are living as parasites at the expense of the cell, the term "phagocytic," as applied to the cell, is apt to be misleading. In retrogressive infections, however, the bacilli which have penetrated endothelial cells undoubtedly perish.

(*c*) CELLS WITH DIFFUSELY STAINING NUCLEI.

It is not always possible to demonstrate a bacillus actually within cells of this character, but in a large number of cases one or more bacilli can be found either within or near at hand. It seems probable that this change in the cell is attributable to the local irritant action of the bacillus. Cells showing this modification of their nuclear material only occur here and there, and in this respect may be contrasted with the merely swollen type of endothelial cell, which is often generally distributed.

(*d*) MULTINUCLEATE CELLS.

When the endothelial nucleus undergoes changes leading to direct division, bacilli are frequently found either in immediate contact with it or near at hand. This association is sufficiently constant to justify the assumption that the nuclear changes are due to the action of the bacillus. From the earliest stage of commencing division down to the latest stage where the nuclei form aggregations characteristic of giant cells, this association between bacilli and nuclei is observable. And in the protoplasm in which the nuclei are embedded the bacilli are often numerous and, not infrequently, appear to have increased by multiplication. These structures, therefore, do not appear to be "phagocytic," in the sense that they devour the bacilli; they appear rather to be more or less favourable for the conservation and increase of the bacilli. In chronic or retrogressive lesions where bacilli are difficult to find, it commonly happens that the only bacilli discoverable occur in these situations, though, of course, when bacilli are dying out they perish here as well as elsewhere.

(*e*) PROLIFERATING CELLS.

In local lesions removed a short time after the subcutaneous inoculation of a large dose of bacilli, one or two bacilli may occasionally be found in an endothelial cell showing mitotic figures. But this condition is exceptional and is probably due to the accidental arrival of a bacillus in a cell which happens to be undergoing mitosis. Owing to the strong evidence that the infection of the tubercle bacillus interferes with the normal vitality of an endothelial cell, it is not likely that the mitosis would be followed by the production of normally vigorous daughter cells. And in situations showing newly formed endothelium, bacilli may be found in proximity to these cells, but they are rarely found within them. But it sometimes happens that when a new vascular channel is in process of formation and penetrates into a site where bacilli are present, a fresh passage is afforded for the transference of bacilli into the circulation.

(*f*) DEGENERATING CELLS.

Intact capillaries are frequently found in caseating foci. The endothelial walls of these channels degenerate sooner or later, and before they break down they often contain bacilli. When a break in their continuity occurs, the opportunity is offered for bacilli, whether within the endothelial cells or in the external caseous tissue, to gain access to the circulation.

E.—THE RELATION OF PLASMA CELLS TO MAMMALIAN TUBERCLE BACILLI.

(1.) Infections during the First Fortnight.

AFTER TWENTY-FOUR HOURS.

In the local lesion of Calf 438, killed 24 hours after subcutaneous inoculation, there are no signs of plasma cells.

In two rabbits killed at the same period, after intravenous inoculation, there are a few cells the protoplasm of which takes a pyronin stain both in the lungs and in the livers, but these cells are scanty and do not form groups. In the lungs they are sometimes found amongst the loose connective tissue surrounding the bronchioles, and in the livers they are present in the interlobular connective tissue.

The lungs of Pig 144 killed 24 hours after subcutaneous inoculation show plasma cells scattered about in all parts of the section ; they do not form groups of any size, but are most numerous in the neighbourhood of bronchioles. Many of the cells show mitotic figures.

AFTER THREE DAYS.

In the local lesions of Calves 284 and 873 no plasma cells have been found. Specially stained specimens of the lungs and livers also failed to show plasma cells.

In two rabbits killed at the same period, after intravenous inoculation, the lungs and livers show a few suspicious cells taking a pyronin stain, as in the 24 hours' specimens, but in none of these cells have mitotic figures been found.

The lungs of Pig 146 killed three days after subcutaneous inoculation show a distribution of plasma cells similar to that found in the 24 hours' pig ; mitotic figures are found but are not more numerous than in the former specimen.

AFTER FIVE DAYS.

In the lungs of two rabbits, killed after intravenous inoculation, there is an increase, compared with the three days' rabbits, of the number of cells reacting to Pappenheim's stain ; many of the cells show mitotic figures. Some of the cells are small and fusiform, others are about the size of large lymphocytes ; they do not form groups. In the interlobular connective tissue of the livers there are a few cells which take up the pyronin stain, but none have been found in mitotic division.

In the lungs of Pig 148 killed five days after subcutaneous inoculation the plasma cells show a considerable increase as compared with the three days' specimen and have a tendency to collect in groups.

AFTER EIGHT DAYS.

In the local lesion of Calf 292, killed after subcutaneous inoculation, groups of plasma cells are found at the margin of necrotic areas ; some of these cells are in mitotic division. In Calf 881, killed at the same period, the local lesion shows cells which appear to be of the same nature, but they do not stain well. In the lungs of Calf 292 plasma cells are numerous, particularly at the periphery of bronchioles and blood-vessels, where they often form definite rings. Cells of the same nature also occur in many places in the alveolar walls. These cells are also present in the early tubercles found in this specimen, but not accumulated in large numbers. Many of the cells are in mitotic division. In the liver of the same animal plasma cells are found both amongst the interlobular connective tissue and occasionally within the lobules ; they are much rarer than in the lungs ; no mitotic figures have been observed.

In the lungs of Pig 142, killed after subcutaneous inoculation, plasma cells are numerous and are generally distributed ; they are conspicuous in the early tubercles but are more numerous round the margins than in the interior. Mitotic figures are numerous. In the liver of the same animal plasma cells are present in the early tubercles, being more numerous at the periphery than in the centre ; they also occur in the interlobular tissue and occasionally within the lobules. On the whole they are scantier than in the lungs and fewer of them show mitotic figures.

AFTER TEN DAYS.

The lungs and livers of two rabbits, killed after intravenous inoculation, were found to be in a condition of highly acute infection. There is no marked increase in the number of plasma cells, compared with the five days' rabbits ; and, as often happens in acute infections, the cells which appear to be of this nature do not react well to the stain. The kidneys of the same animals are only slightly infected and contain no definite plasma cells.

AFTER FOURTEEN DAYS.

In the local lesion of Calf 288, killed 14 days after subcutaneous inoculation, plasma cells show no increase as compared with the corresponding eight days' specimen, and many of those present are degenerating. In the local lesion of Calf 869, killed 14 days after inoculation with bacilli of much lower virulence, plasma cells are far more numerous than in the lesion of Calf 288 and frequently show mitotic figures. In the lungs of Calf 288 there are numerous tubercles and the disease is in a more advanced condition than in the lungs of the corresponding eight days' animal (Calf 292). Compared with the latter animal, the plasma cells, though showing a similar distribution, are decidedly fewer and frequently appear to be breaking down. In the lungs of Calf 869 there are only a few small tubercles ; plasma cells are rare, but a few are present within the tubercles and at the periphery of bronchioles and blood-vessels ; some of them are in mitotic division. In the liver of Calf 288 there are several small tubercles in which plasma cells are present but rare. In the liver of Calf 869 the amount of infection is only slight, but a few plasma cells are present in the interlobular tissue and in association with the lesions. The kidneys of Calf 288 contain several minute lesions in which plasma cells are conspicuous. A few mitotic figures have been noted.

In the lungs of Pig 150, killed 14 days after subcutaneous inoculation, the lesions are larger and more numerous than in the eight days' animal (Pig 142). Plasma cells appear to be about as numerous as in the latter animal, and their general distribution is the same, except that they have disappeared from the centres of the tubercles which are breaking down. Many of the cells show mitotic figures. In the liver the plasma cells are in the same condition as in the liver of the eight days' animal.

(2.) Later Infections—Acute.

In a series of rabbits inoculated intravenously with 1 mg. of highly virulent bacilli the infection proved fatal during the third and fourth weeks. In the animals killed at 15 days plasma cells are more numerous and more typical in character than in the ten days' specimens, both in the lungs and the livers ; they also occur rather more frequently in the kidneys. Mitotic figures are often found. Most of the later cases terminated fatally. In these the number of plasma cells is variable, but in the kidneys, which are less severely affected than the lungs and livers, it shows an increase.

In calves which died, or were killed when moribund, after subcutaneous inoculation plasma cells are always present in the infected tissues, though in some cases, particularly in animals which were found dead, they do not react well to the stain. In the lungs these cells occur (*a*) amongst the areolar tissue surrounding bronchi, bronchioles, arteries, and veins ; (*b*) at the periphery of caseous patches ; (*c*) scattered singly or in small groups amongst the tissue external to the definitely tubercular areas and more particularly in situations which are at the commencement of tubercular change. The plasma cells are not found in any of their characteristic forms within blood-vessels. They have no definite relationship to the normal lymph nodes which are to be found in the lung. These nodes are generally situated close to one side of a bronchus, but the plasma cells are found equally distributed all round the bronchus, and there is no evidence of a migration of plasma cells from these lymph nodes to the various situations where plasma cells occur. The plasma cells found in close proximity to bronchi and blood-vessels are very frequently of the compressed type, resembling in outline connective tissue corpuscles, but with somewhat swollen protoplasm. In most specimens mitotic figures can be found. Within the livers of these acutely infected calves plasma cells occur both within the tubercular areas and amongst the areolar tissue which surrounds the interlobular vessels. Occasionally a cell reacting to Pappenheim's stain is to be found within a blood capillary. The plasma cells are most numerous and conspicuous, and stain most deeply, in the interlobular connective tissue, particularly where this tissue is adjacent to a tubercle; every variety of form is here present, from the large swollen cell to the flattened or elongated. Within the tubercles the plasma cells do not appear able to survive for a long period. They are, however, frequently found even in very early tubercles, and round the margins of those which have advanced to the caseous stage some are almost invariably present. The plasma cells directly in contact with the lesions are not of the compressed type but generally have a swollen protoplasm which often fails to take a deep stain. In the case of tubercles which border upon interlobular connective tissue, it is noticeable that the part of their periphery in contact with this tissue is much richer in plasma cells than the rest of the tubercle, and the general appearance strongly suggests that the plasma cells of the tubercle are derived from the connective tissue spaces but do not survive long after separation from the source which supplies them. Mitotic figures are frequently found. It is noteworthy that in lesions of the acute type plasma cells do not form a definite layer sharply marking off the lesion, and the cells found at the periphery of the lesions show no tendency to pass into fibroblasts. In these acute infections of calves the kidneys are less severely affected than the lungs or liver. In the kidneys plasma cells are invariably present in association with the tubercular areas. The centrally caseous miliary tubercles are usually surrounded by an abundant zone of these cells, and plasma cells are also numerous in the tracts of interstitial infiltration which show evidence of commencing tubercular change. Surrounding the tubercles, the plasma cells are generally rounded or oval in outline; in the interstitial tissue, compressed or elongated forms are more frequent, and the cells which occur apart from obvious lesions are not infrequently like ordinary connective tissue corpuscles, except for the red colour of their protoplasm. Very occasionally, a cell which reacts to Pappenheim's stain is found within a glomerulus. Mitotic figures are not infrequent.

(3.) Later Infections—Less Acute and Chronic.

In passing to lesions where the microscopic evidence shows that the process of tissue destruction is less rapid, an increase in the number of plasma cells can generally be noted. Some of these cells may be found surviving in the more central parts of the less advanced lesions, and at the marginal areas they show a greater tendency to accumulate as a definite layer. Another feature observable, more particularly at the periphery of the larger caseous areas which show a well defined demarcation zone, is that some of the plasma cells, whilst retaining a good pyronin stain, have assumed the shape of fibrous tissue corpuscles. Numerous transitional forms can be observed between the cell with an oval outline, the rather narrower cell with slightly tapering extremities, and the very narrow cell with long, tapering extremities. This is the reverse process to what is found in earlier infections, where, particularly in the tissue external to blood-vessels and bronchioles, transitional forms are commonly found from the narrow to the oval or rounded type of cell. Examples of this intermediate condition are found in the lungs of the following animals:—Calf 312, killed 90 days after subcutaneous inoculation with 50 mg. of a porcine virus; Calf 365, killed 61 days after subcutaneous inoculation with 100 million bacilli of relatively low virulence, present in an emulsion of human material; Calf 905, killed 90 days after subcutaneous inoculation with 50 mg. of bacilli possessing low or intermediate virulence; Goat 10, killed 165 days after subcutaneous inoculation with 4½ million bovine bacilli; Goat 28, died 91 days after subcutaneous inoculation with ·01 mg. of bovine culture.

In lesions of more chronic or more definitely retrogressive types, the numbers and characters of the plasma cells vary according to the extent and nature of the destructive changes which have been produced. In slight lesions, attributable to very small deposits of bacilli which have found themselves unable to multiply, there is little or no caseation, but perhaps one or two giant cells may be found. In lesions of this nature plasma cells are frequently found; but they are not abundant; they are distributed irregularly in various parts of the focus and do not form a definite boundary layer. It is evident that the tissue disturbance has not been sufficiently great to stimulate a large production of plasma cells. In lesions of a definitely chronic type, but containing a central area of completely caseous or caseo-calcareous material, the plasma cells vary according to the stage of retrogression which has been reached. When the peripheral zone of the tubercle contains many cellular elements of a non-fibrous nature, the plasma cell reaction is generally well marked, and these cells form a considerable portion of the cellular element in the peripheral zone. Stages of transition into fibrous elements are much more frequent than in lesions of the intermediate type, and often the majority of the plasma cells have assumed the narrow elongated form. At a more advanced period of retrogression, when the fibrous element predominates in the peripheral zone, cells giving the protoplasmic reaction of plasma cells are much less numerous, and it appears probable that many of the cells which would have given this reaction at an earlier stage have now resolved themselves into ordinary fibrous tissue.

(4.) Relation to other Cellular Elements.

In tissues showing a progressive infiltration of cells derived from the blood, it is usually found that the blood-vessels through which the section passes contain these cells in numbers in excess of the normal. This is the case, for example, in leucocytic infiltrations unless the tissues happen to be removed at a time when the process of immigration is not active. But in examining sections showing various stages in the appearance of plasma cells, it is a significant fact that at no stage is it found that cells which could possibly be mistaken for plasma cells are at all numerous in the blood channels. The only cells not distinguishable with certainty from plasma cells are the large lymphocytes. These cells are sometimes to be found here and there in vessels permeating tissues where plasma cells are demonstrable. But their number is so small and the association of their presence with the occurrence of plasma cells in the tissues is so inconstant, that it is quite impossible to assume that the latter cells have been conveyed by the blood stream. Another very strong piece of evidence in favour of the local origin of the plasma cell is the fact that whenever these cells are at all numerous, especially in the case of developing lesions, mitotic figures are to be found, and frequently a very considerable number of the cells taking the Pappenheim stain show mitoses. The very early appearance of the plasma cells in the connective tissue spaces clearly points to these situations as the sites of origin.

In association with the lesions found in infected lymphatic glands, cells are found which are in every respect identical with the plasma cells found in tubercles occurring in other organs; and as regards the variations in the numbers, morphology, and relationships of these cells in accordance with the severity of the lesions, the lymphatic glands show a correspondence with other organs. Here also the plasma cells are undoubtedly produced *in situ*, and the large number of mitoses found in developing lesions affords strong evidence that this production is actively going on. But owing to the fact that the large lymphocyte normally found in the lymphatic gland takes the characteristic staining reaction of the plasma cells, it is impossible in the lymphatic gland to discover any specially distinctive features of those cells from which cells showing the typical and varied morphological features of plasma cells afterwards develop.

Plasma cells do not form giant cells. Their nuclei, with rare exceptions, show no tendency to divide amitotically; and the constant characteristic of the giant cell is that it contains a conglomeration of nuclei which are the result of direct fission. In Pappenheim specimens the majority of the giant cells show no trace of the pyronin stain; some of them give a slight reddish stain; and occasionally a giant cell is found which shows a deeper reddish streak in some part of its protoplasm. These two latter conditions are readily explained when it is remembered that the protoplasm of a giant cell frequently consists of an amalgamation of protoplasm derived from various cells; to this heterogeneous mixture broken down plasma cells may undoubtedly supply an accidental contribution.

(5.) Relation to Bacilli.

Plasma cells are not phagocytic. They are never found to contain bacilli except when breaking down and imperfectly stained. From areas where bacilli are numerous they rapidly disappear. Though attracted to early lesions and to the periphery of older lesions they do not come into immediate contact with the bacilli. Their appearance may be attributed to some diffusible stimulus associated with the infection.

F.—THE RELATION OF EPITHELIAL CELLS TO MAMMALIAN TUBERCLE BACILLI.

(1.) Pulmonary Epithelium.

In the lungs of calves subcutaneously inoculated with virulent bacilli, in sufficiently large dose to produce rapid dissemination, the first change observable is a very slight thickening, at rare intervals, in the alveolar walls; were it not for the occasional presence of bacilli in these sites, this change is so slight that it would not be considered to be of pathological significance. This condition is found in Calf 284, three days after inoculation. During the second week progressive changes are observable. In Calf 292, eight days after inoculation, the patches of thickening in the alveolar walls are more numerous and the thickening is rather greater. But the lesions have extended mainly by spreading along the course of the alveolar walls, thus tending to surround the alveoli rather than to bulge into their interior. Where the section passes more or less through the centre of an alveolus, showing the alveolar wall in transverse section, the epithelial lining on either side is seen to be slightly swollen, and the nuclei of some of the cells stain more deeply than usual, as though preparing to divide. But where the section meets the circumference of the alveolus more or less tangentially a much larger cellular area is obtained, showing what appears to be an indiscriminate arrangement of epithelial cells, endothelium, and blood capillaries. In a 14 days' specimen (Calf 288) the same condition is observed, the only difference being that the infiltration of the alveolar walls has increased in amount.

These two specimens illustrate an important difference between early tubercles in the lungs and early tubercles in a solid organ such as the liver. A tubercle originating, for example, in the liver parenchyma, expands more or less equally in all directions, and consequently in whatever plane the section happens to pass through it there is the same general relationship of parts, the central portion of the lesion being older than the peripheral. But early tubercles in the lungs are less solid formations; they creep along the alveolar walls and, for a time, only encroach to a slight extent upon the lumen of the alveolus. A section passing diametrically through the alveolar wall shows, in order, swollen epithelial cells, endothelial cells which are also swollen and have lost their distinctness of outline, imperfectly defined blood-capillaries; and tubercle bacilli are seen in direct contact both with endothelial and with epithelial cells. But where alveoli in the same pathological condition are cut tangentially, the lesions occupy a much larger area, though revealing no fresh constituents; instead of being cut across, the elements of the alveolar wall are observed in continuity; the elements which happen to be in the centre of such an area are not the centre of a uniformly expanding focus and may not be in a more advanced stage of pathological change than the elements at the periphery. Areas such as these are constantly encountered in early lesions of the lungs, and, though they may be spoken of as early tubercles, it is important to bear in mind that they are not to be interpreted as though they were sections of solid and more or less spherical nodules.

The following examples may be quoted:—

Calf 436 died 16 days after subcutaneous inoculation with virulent bacilli. The lungs show many small lesions containing numerous bacilli, but the amount of disease is not sufficient to account for death. The epithelial cells are in much the same condition as in the 14 days' animal (Calf 288). They are swollen, and their nuclei sometimes show signs of division, but the majority of the cells still retain their connection with the alveolar walls, and desquamated cells are not common in the alveoli.

Calf 258 died 18 days after subcutaneous inoculation, and the lungs show a sufficient amount of disease to account for death. The swelling up of the epithelial cells is very conspicuous and, as contrasted with the previous animals, large numbers of these cells are desquamating into the alveoli. Both completely detached cells and swollen cells protruding into the alveoli are found in all parts of the specimen. Some of the cells show mitotic divisions; in a larger number direct division of the nucleus is in process. The same conspicuous swelling up and desquamation of the epithelial cells have been found in other calves in which subcutaneous inoculation proved fatal during the third week, *e.g.* in Calves 244, 254, 376, 933 (19 days) and Calf 569 (20 days). In these specimens also mitotic figures are occasionally met with but indications of direct divisions are much more frequent.

From the third week onwards all the acutely infected calves' lungs show the same condition, viz., swelling up and copious desquamation of the epithelial cells, abundant evidence of amitotic division and some signs of mitotic division, or of abortive attempts at mitosis.

Concurrently with this filling up of the alveoli with desquamated cells, the infection of the alveolar walls advances, and the tubercle then becomes a solid body in which the distinction between the walls and the contents of the alveoli gradually becomes obliterated. When caseous degeneration is progressing rapidly, the epithelial cells quickly disappear from the central parts of the lesions. Where the destructive process is less acute, the appearance of the epithelial cells varies according to whether or no they have retained the continuity of their protoplasmic outline. In the former case, these cells, greatly swollen and staining rather faintly, may form groups; the individual cells are separated from each other by a polygonal outline, and the general appearance and arrangement resembles a layer of epithelium; hence the term "epithelioid," which is sometimes applied to those cells arranged in this manner. But more commonly, in acute infections, the epithelial cells which have become incorporated into a tubercle fail to preserve their individuality, and their protoplasm gradually becomes fused with the protoplasm of adjacent endothelial or other cells; at this stage there is a matrix of protoplasm in which nuclei are embedded; to both nuclei and protoplasm epithelial cells contribute, but their identity is soon lost.

Epithelial cells readily take up bacilli, and the swollen type of cell, particularly the desquamated cell lying free within an alveolus frequently contains several; the bacilli appear to find the interior of these cells favourable for multiplication.

In acute infections of the pig by subcutaneous inoculation the lung epithelium follows the same changes as in the calf. Thus in Pigs 142 and 150, killed 8 and 14 days after inoculation, there is a similar infiltration of the alveolar walls, together with some swelling of the epithelial cells and occasional signs of nuclear division, but with little or no desquamation of the cells and escape into the alveoli. The lungs of Pig 94, killed 61 days after subcutaneous inoculation with 1 mg. of virulent bacilli, show acute disease at a later stage. In this specimen there is very abundant desquamation of epithelial cells and the alveoli filled with this material are in process of fusion with the adjacent tubercular areas. In the lungs of Pig 80, killed 131 days after feeding with bacilli, the tubercles in the lungs are expanding in the same way, by fusion with adjacent alveoli filled with desquamated cells, and where the alveolar boundaries have disappeared the "epithelioid" arrangement of the cells is well shown. In this specimen several mitotic figures are found, though, as is usually the case, amitotic division is much more common. Bacilli are scanty in this specimen, but in Pig 94, where they are much more numerous, they occur in considerable numbers within desquamated epithelial cells.

After intravenous inoculation swelling up of the epithelial cells is more rapid and is more promptly followed by desquamation. In rabbits this swelling up of the cells is well marked in 24 hours, and is still more conspicuous in three days; in both the 24 hours' and the three days' specimens a few cells have become detached and escaped into the alveoli. In the five days' specimens the number of desquamated cells has greatly increased. In the 10 days' specimens there is a further advance in the process; in some of the cells the nucleus has divided into two, and in many places a small group of cells has partially fused together. In 15 days there is further swelling of the cells and amitotic division of the nuclei, and many of the swollen epithelial cells are partially fused together. In one specimen, showing advanced consolidation, with many caseous areas, this feature is particularly prominent. In areas where the alveoli are not completely occluded, desquamated alveolar cells are often found in groups, and in many instances there is complete fusion of their protoplasm, whilst their nuclei are arranged peripherally. Within the protoplasm tubercle bacilli are generally to be found. Passing into completely consolidated areas, similar appearances are seen. The cell fusions here described are irregular rather than circular in outline, and generally the nuclei are not equally distributed at all parts of their periphery. There are also found, lying free within alveoli which are not completely consolidated, what at first sight appear to be tubes cut transversely and filled with coagulated deposit. On closer examination, the central part of many of these is found to consist of a greatly swollen alveolar cell; this is surrounded by nuclei apparently derived from other cells which have wrapped themselves round the epithelial cell whilst their protoplasm has fused with the protoplasm in the central area.

The lungs of calves, like those of rabbits, show a rapid and profuse swelling up and desquamation of epithelial cells after intravenous inoculation.

In less acute infections the condition of the epithelial cells varies with the type of the lesions. In the retrogressive type exhibiting a caseous centre surrounded by a broad fibrous zone, it may be impossible to recognise any survival of the alveolar epithelium within the tubercles, and in the surrounding tissue this epithelium may appear normal. In circumscribed tubercles where the fibrous element is less developed swollen epithelial cells may participate in the formation of the so-called epithelioid zone; and in lesions which are progressing, though slowly, the tissue surrounding the tubercles shows a swelling up and desquamation of the alveolar cells which, though localised, is comparable in character to the general condition observed in acute infections. In very mild infections where retrogression has set in after no more than a slight amount of caseation has been produced, the lesions are generally overrun with lymphocytes and other wandering cells and very few epithelial cells can be made out; it is also difficult to trace the origin of any giant cells which may be present, though from a comparison with more acute lesions showing these structures it appears probable that the disintegrated epithelium has contributed to their origin.

(2.) Epithelium of Secretory Glands.

In the secretory glands possessing a characteristic epithelium, this structure usually takes a less active part than the pulmonary epithelium in the process of tubercle formation.

LIVER.

In searching specimens illustrating early dissemination of tubercle bacilli in the liver a bacillus is occasionally found within a parenchymatous cell which appears to be histologically normal. But these instances are rare and do not invalidate the general rule that the presence of tubercle bacilli at once sets up pathological changes in the liver cells with which they come into contact. The cells fail to take their normal protoplasmic stain, and their nuclei lose their regularity of contour. Occasionally a mitotic figure may be found in a liver cell which is close to a tubercular focus, or the nuclear chromatin may assume a deeper stain suggestive of preparation for mitosis; but these indications of normal reproduction are exceptional.

It is, however, erroneous to assume that the liver cells disappear *in toto* and are replaced by entirely new elements. In early tubercles, for example in the calf, pig, and rabbit, the first change noted is that the protoplasm of the liver cell loses its outline and merges with the protoplasm of adjacent endothelial and parenchymatous cells. Closely following this change there is often, but not always, an alteration in the shape of the nucleus. When this stage is reached the liver cell has lost its individuality and is merged into a fusion of protoplasm containing nuclei. Many of these nuclei divide by direct division; and it frequently happens that the nuclei of the parenchymatous cells contribute to these daughter nuclei, since these cells often show signs of direct division before they have lost their individuality as separate cells. It is also commonly found, particularly at the margin of an area commencing to caseate, that the nuclei retain their normal shape after the protoplasm has commenced to disintegrate; and such an arrangement of large nuclei regularly disposed in an imperfectly defined protoplasmic area often contributes to an "epithelioid" stage of tubercle formation.

In examining the groups of newly formed nuclei which are characteristic of early tubercles, the endothelial and parenchymatous elements are often so intimately blended that it is impossible to assign to these nuclei a distinctive origin from one or the other type of cell; but in early lesions clear evidence may frequently be found that the nuclei of the liver cells play an important part in the process and that they possess a remarkable capacity for multiplying by direct division. This is well shown in the livers of rabbits killed in early stages after intravenous inoculation.

In these specimens the nuclei often form large masses, occupying a central area which is surrounded by the fused protoplasm of two or three liver cells. In many of the lesions where these large giant cells occur the endothelial element is inconspicuous, and though the possibility of endothelial elements having been incorporated in the cell mass cannot be excluded, there is clear evidence that the parenchymatous cells have contributed largely, if not exclusively, to both the nuclei and the protoplasm of many of the giant cells. In another animal (Rabbit 1283) killed 29 days after intravenous inoculation with 50 mg. of dead bacilli, the liver shows an extremely large number of small giant cells, in which it is evident that the protoplasm has been formed by the fusion of adjacent parenchymatous cells. In this case the nuclei of the giant cells appear to be derived partly but not exclusively from the parenchymatous cells, the other source from which the nuclei have been derived being the capillary endothelium. And an additional feature of interest in this specimen is the invasion of giant cells by small lymphocytes.

KIDNEY.

The part played by the epithelium of the renal tubules in tubercle formation is relatively small. These cells rarely contain bacilli, except when disintegrated, and they show no sign of multiplication when in contact with developing tubercular foci. As the tubercular process spreads along the interstitial tissue the tubules are gradually cut off and may often be seen in section within well formed tubercles. The degenerating cells of the renal epithelium then often present some resemblance to giant cells, but their nuclei show no signs of multiplication by direct division, and there is no evidence that the multinucleate mass which constitutes a genuine giant cell is derived from the renal epithelium.

MAMMARY GLAND.

Like the renal epithelium, the cells lining the mammary tubules do not take an active part in tubercle formation. When degenerating, these cells may contain bacilli, and in cells which have been shed and carried into the collecting tubes bacilli are often numerous. But the usual process of infection is a progressive invasion of the interstitial tissue with a gradual invasion and breaking down of the glandular cells, when these have been cut off from their normal surroundings. Mitotic figures are sometimes found in epithelial cells, but too rarely to indicate that the invasion of bacilli has stimulated proliferation. Within definitely formed tubercles the mammary cells speedily disappear.

LESIONS PRODUCED BY AVIAN TUBERCLE BACILLI.

I.—LESIONS IN FOWLS AND PARROTS.

(a) INTRAVENOUS INOCULATIONS IN FOWLS.

(1.)

VIRUS—P III.

Animal Inoculated.—Fowl 100.

Fowl 100 was inoculated intravenously with 1 mg. of culture of P III (a porcine virus with avian characters), and died 33 days afterwards.

Lungs.

There are several small foci which consist of colonies of bacilli surrounded by fibrous tissue. Loosely distributed bacilli also occur in many parts of the tissue, but these organisms have not grown into colonies ; in some situations they are surrounded with oxyphil leucocytes and the remains of these cells ; in other places bacilli appear to be within or adherent to the capillary walls. The whole of the tissue contains large numbers of oxyphil leucocytes. This is the most prominent histological change.

Liver.

The tissue is packed with clusters of bacilli. At the periphery of each of these groups of bacilli there are several layers of fibrous tissue and an abundant deposit of hyaline material. The nodules thus formed are more or less circular in outline and have replaced nearly the whole of the liver tissue. Their interior is not caseous but contains cells which stain badly, and appear to be swollen and distorted parenchymatous and endothelial cells. No giant cells are present. In the interstices of tissue left between the nodules plasma cells are numerous, and there is a moderate sprinkling of oxyphil leucocytes.

Kidneys.

With the exception of one minute group of bacilli which have produced no definite lesion, the specimen examined is normal.

Spleen.

The spleen is in the same condition as the liver.

SUMMARY.

The lungs contain several small groups of bacilli which are surrounded by fibroblasts. The liver is a mass of small nodules which have a broad periphery of fibrous and hyaline material and are filled with bacilli. The kidneys appear to have almost completely escaped infection.

(2.)

VIRUS—P XXI.

Animal Inoculated.—Fowl 152.

Fowl 152 was inoculated intravenously with 1 mg. of culture of P XXI (a porcine virus with avian characters), and was killed, when ill, 42 days afterwards.

Lungs.

Small groups and colonies of bacilli are scattered throughout the tissue. The bacilli have produced very little tissue reaction, with the exception of one group which are enclosed within a fibrous nodule. The bacilli forming this group are long (about 4 μ) and more or less curved ; in other parts of the tissue the bacilli are very short and straight.

Liver.

The tissue is crowded with bacilli, which form colonies showing some tendency to be surrounded by fibroblasts.

Kidneys.

Several small colonies of bacilli are present.

Spleen.

Like the liver, the spleen is crowded with large colonies of bacilli.

SUMMARY.

Severe infection of liver and spleen ; scattered colonies of bacilli in the lungs and a few in the kidneys.

(3.)

VIRUS—P XLIV.

Animal Inoculated.—Fowl 172.

Fowl 172 was inoculated intravenously with ·01 mg. of culture of P XLIV (a porcine virus with avian characters), and died 56 days afterwards.

Lungs.

There are a few slightly caseous patches in which bacilli are abundant, the rest of the tissue being normal.

Liver.

The liver contains very large numbers of bacilli, which have grown into colonies and rosettes. The bacilli have produced many caseous areas, some of which are discrete, whilst others are semi-confluent. Associated with the lesions multi-nucleate masses and giant cells are generally found.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Bacilli are even more abundant than in the liver. The tissue is riddled with large, confluent, caseous foci. Aggregations of nuclei are present, but are less conspicuous than in the liver.

SUMMARY.

In the liver and spleen the bacilli have multiplied very copiously and have produced extensive tissue destruction. The lungs are only slightly affected.

(4.)

VIRUS—A II.

Animal Inoculated.—Fowl 92.

Fowl 92 received an intravenous inoculation with 0·1 mg. of culture of A II, and died 29 days afterwards.

Lungs.

Throughout the tissue there are numerous small lesions characterised by partial cell necrosis and a more or less definite fibrous boundary. In all these situations bacilli are numerous and in many of them have grown into semi-solid clumps; there is some indication of a tendency to rosette formation. Bacilli do not occur apart from these foci, which presumably have originated from bacilli deposited shortly after inoculation. The tissue is intensely engorged with red blood-corpuscles. On this account, and perhaps also owing to some post-mortem change, the demonstration of oxyphil leucocytes has not been successful; judging from the characters of the nuclei of the cells in the semi-necrotic areas, these cells are not numerous. The bacilli are short, straight, and uniformly stained.

Liver.

The entire tissue is crowded with groups of bacilli; in most of the groups the rosette formation of the bacilli is well marked. In the areas occupied by the bacilli the tissue cells are partially necrotic. There is neither caseation, fibrous demarcation of the foci, nor giant cell formation. There is a general infiltration of the tissue with leucocytes and lymphocytes.

Kidneys.

No lesions have been found, but a few bacilli are present in blood-vessels.

Spleen.

Like the liver, the spleen is densely packed with colonies of bacilli. The bacilli have produced partial necrosis, but have not led to giant cell formation. There is no evidence of fibrous demarcation of the areas in which they occur.

SUMMARY.

The liver and spleen are swarming with bacilli. The organisms are evidently growing much more freely here than in the lungs. In the lungs they are much less abundant, and the foci which contain them are generally surrounded by fibrous tissue. The kidneys seem to have escaped infection.

(5.)

VIRUS—A II.

Animal Inoculated.—Fowl 96.

Fowl 96 was inoculated intravenously with 10 mg. of culture of A II, and died 37 days afterwards.

Lungs.

The tissue is engorged with red blood-corpuscles, but there is no histological change indicative of tuberculosis. Under a low power the whole of the tissue is seen to be sprinkled with colonies of bacilli. Some of the colonies have irregular gaps in the middle, but they do not form well marked rosettes. Under a higher magnification many smaller groups of bacilli are also found. Several bacilli have been seen within the blood-vessels. The distribution of bacilli is quite irregular, and their presence does not seem to have provoked any tissue reaction. No fibrin is present. The bacilli are straight, uniformly stained, and very small, generally from ·5 to 1 μ in length and not often longer than 1 μ.

Liver.

In sections not stained for bacilli the tissue is riddled with small, pale areas, between which the unaltered and more deeply stained cells of the tissue appear like the meshes of a network. The pale areas consist of swollen and distorted parenchymatous and endothelial cells. These areas do not present any particular structure characteristic of tubercles. No giant cells are present. There is no fibrin. In carbol fuchsin and methylene blue specimens tubercle bacilli are so numerous that they appear to occupy almost as much space as the tissue cells; they correspond, in situation, to the pale areas referred to above. The bacilli have grown in confused masses and colonies, with only a slight tendency to rosette formation. Though often present within the protoplasm of the cells, the bacilli are not characterised by any special limitation to, or preference for, the interior of cells. The length of the bacilli is rather greater than in the lungs, forms as long as 2 μ being frequent; they are nearly all straight and uniformly stained.

Kidneys.

No histological lesions are noticeable, but there are several small clusters of bacilli. The bacilli occur in all parts of the tissue and not infrequently occupy glomeruli. Compared with the liver, the number of bacilli present is very small.

Spleen.

In the spleen colonies of bacilli are even more numerous than in the liver. They form red masses occupying more than half the total area of the section. Rosette forms are numerous. The amount of tissue destruction is very small, relatively to the enormous number of bacilli present.

Bone Marrow.

Numerous small clumps of bacilli are present. The bacilli are very minute, often not more than ·5 μ in length, and have produced foci of partial necrosis. No fibrin is demonstrable.

SUMMARY.

General dissemination of bacilli, which have multiplied to an enormous extent, particularly in the spleen and liver. Very little tissue destruction. No formation of tubercles.

(6.)

VIRUS—A IV.

Animal Inoculated.—Fowl 108.

Fowl 108 received an intravenous inoculation with 50 mg. of culture A IV, and died 35 days afterwards.

Lungs.

Bacilli, forming small solid colonies and groups of colonies, aae scattered throughout the tissue. Many of the larger groups are surrounded by a scanty zone of fibroblasts. Oxyphil leucocytes are not abundant. The bacilli are minute, straight, and uniformly stained.

Liver.

The tissue consists of a mass of clusters of bacilli, separated from each other by a relatively small amount of liver tissue. The size of each cluster is from about one to four times that of a renal glomerulus. Surrounding the great majority of the groups of bacilli there is a scanty peripheral layer of fibroblasts.

Kidneys.

Within a few glomeruli fairly large groups of bacilli are present, and minute groups are formed in many parts of the tissue. Very little tissue change has been produced.

Spleen.

Like the liver, the spleen is occupied everywhere by dense masses of bacilli. In some instances, but not so frequently as in the liver, the groups of bacilli are surrounded by fibroblasts.

SUMMARY.

The liver and spleen are almost solid with bacilli. The lungs are less severely affected and the kidneys only slightly.

(*b*) FEEDING EXPERIMENTS IN FOWLS.

(1.)

VIRUS—P II.

Animal Fed.—Fowl 32.

Fowl 32 was fed with two glycerin-serum cultures of P II (a porcine virus with avian characters), and died 107 days afterwards.

Lungs.

A few suspicious cell fusions, hardly typical of giant cells, are present, but no tubercle bacilli.

Liver.

Two specimens of liver have been examined. In one the only abnormality noted is a small amount of cellular infiltration at the periphery of a few of the larger blood vessels. In the other there are broader zones of infiltration at the periphery of larger blood vessels, and in two of them there are patches of necrosis and groups of cell fusions, more or less resembling giant cells. In neither specimen have any tubercle bacilli been found.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The spleen contains large areas which are extensively caseous and contain numerous giant cells. Bacilli are distributed in fairly large numbers throughout these areas, and are generally present in groups within the giant cells.

SUMMARY.

Except in the spleen, which is extensively affected, only a slight amount of disease has been produced in the organs examined. The bacilli have nowhere grown into dense colonies.

(2.)

VIRUS—P III.

Animal Fed.—Fowl 36.

Fowl 36 was fed with a whole glycerin-serum culture of P III (a porcine virus with avian characteristics), and died 96 days afterwards.

Lungs.

In the specimen examined there is one small lesion consisting of a semi-necrotic area surrounded by leucocytes and lymphocytes. In this area a few bacilli and fragments of bacilli are present; none have been found elsewhere.

Liver.

The liver contains many caseous foci which have a periphery of fibroblasts, infiltrated by leucocytes. In the areas where caseation is commencing, there are aggregations of cell nuclei, but no typical giant cells. Bacilli occur both in the centre and at the periphery of the caseous foci, and sometimes form groups and occasionally small colonies. The bacilli vary in length from 1·5 to 4 μ and are often curved and beaded.

Kidneys and Spleen.

No lesions or bacilli have been found.

SUMMARY.

Extensive infection of the liver.

(3.)

VIRUS—P XXI.

Animal Fed.—Fowl 26.

Fowl 26 was fed with 10 mg. of culture of P XXI (a porcine virus with avian characters), and was killed 92 days afterwards.

Lungs.

A few suspicious cell fusions have been found, but no tubercle bacilli.

Liver.

Surrounding some of the larger blood-vessels and bile ducts there is a broad zone of infiltration consisting of dense masses of oxyphil leucocytes together with many lymphocytes and plasma cells. Near the same situations there are also many circular islands which are densely packed with leucocytes and lymphocytes and have a slight peripheral margin of fibroblasts. In some of these islands the lymphocytes preponderate, in others the leucocytes. In one of these islands, which is distinguished from the rest by being slightly necrotic in the centre and containing, together with the remains of liver cells, many leucocytes but no lymphocytes, a few tubercle bacilli are present. No bacilli have been found in any other situation. In addition to the above mentioned situations, many small aggregations of leucocytes and lymphocytes are present within the lobules; it is noteworthy that these frequently occur close to the periphery of blood-vessels. In the single lesion where bacilli are demonstrable, these organisms appear to be in process of destruction; it is probable that they would have soon disappeared and that then this lesion would, like the others, have become completely filled up with leucocytes and lymphocytes. Possibly in many, if not all, the other lesions observed, tubercle bacilli were present at an earlier date.

Kidneys.

No lesions or tubercle bacilli have been found.

Spleen.

Caseous tubercles are present. They are bounded by a well marked fibrous zone; internal to this is a ring of giant cells; the protoplasmic outlines of the giant cells are indistinct, and their nuclei are characterised by being heaped up in the centre of the cell. Masses of broken down leucocytes are found amongst the caseous material of some of the tubercles. Bacilli are present in the tubercles but are rare.

SUMMARY.

After feeding with a small dose (10 mg.), definite, but retrogressive, infection has been produced in the liver and spleen; and in the latter organ there are many fibro-caseous tubercles containing numerous giant cells.

(4.)

VIRUS—P XXI.

Animal Fed.—Fowl 28.

Fowl 28 was fed with 10 mg. of culture of P XXI (a porcine virus with avian characters), and died 103 days afterwards.

Lungs.

No lesions or bacilli have been found.

Liver.

There is a marked leucocytic infiltration, particularly around the blood-vessels and between the lobules; a few tubercle bacilli occur, but there are no definite tubercles.

Spleen.

A few small, caseous foci are present and contain a few bacilli.

Cervical Gland.

There are a few small groups of bacilli, associated with slight necrotic change.

SUMMARY.

Slight infection of liver, spleen, and a cervical gland; bacilli scanty.

(5.)

VIRUS—P XXVII.

Animal Fed.—Fowl 40.

Fowl 40 was fed with one glycerin-serum tube of culture of P XXVII (a porcine virus with avian characters), and died 63 days afterwards.

Lungs.

There are a few small foci, each containing a group of leucocytes and lymphocytes; in one of these situations a tubercle bacillus has been found.

Liver.

There is a leucocytic infiltration, particularly well marked near the blood-vessels, and one patch of slight caseation has been noted; no tubercle bacilli have been found.

Spleen.

No lesions or bacilli have been found.

SUMMARY.

Very slight infection.

(6.)

VIRUS—A I.

Animal Fed.—Fowl 146.

Fowl 146 was fed once with 10 mg. of culture of A I, and died 131 days afterwards.

Lungs.

No lesions or bacilli have been found.

Liver.

The specimen contains a few isolated, caseous foci which have a broad periphery of lymphocytes, leucocytes and fibroblasts, and internal to this a zone of giant cells. Bacilli are very scanty.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are several caseous areas with no definite demarcation zone. Bacilli are very rare.

SUMMARY.

Isolated, necrotic lesions, containing very few bacilli, are present in the liver and spleen.

(7.)

VIRUS—A X.

Animal Fed—Fowl 264.

Fowl 264 was fed with the tissues of a rat infected with A X, and was killed 162 days afterwards.

Lungs.

No lesions or bacilli have been found.

Liver.

The specimen contains a large lesion which is completely necrotic in the centre and is surrounded by a well-defined ring of fibroblasts, outside which there is a broad zone of lymphocytes and leucocytes. There is also a well marked infiltration of lymphocytes and leucocytes at the periphery of the blood-vessels. A few bacilli are scattered about the necrotic centre of the lesion, but none have been found elsewhere.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There is one large caseous patch, in which bacilli are scanty.

Intestine.

A portion of the intestinal wall is greatly thickened and contains large caseous tracts. Bacilli are fairly plentiful.

SUMMARY.

The liver and spleen contain isolated caseous lesions in which bacilli are scanty.

(c) SUBCUTANEOUS INOCULATIONS IN FOWLS.

(1.)

VIRUS—P II.

Animal Inoculated.—Fowl 158.

Fowl 158 was inoculated subcutaneously with 1 mg. of culture of P II (a porcine virus with avian characters), and died 108 days afterwards.

Lungs.

There are many large nodules which are in an advanced stage of caseation and contain large clusters of bacilli. At the periphery of the nodules there is a zone of fibroblasts. Many of the bacilli are long and curved.

Liver.

Very little normal tissue is left. There are large tracts of completely caseous material and also smaller foci which are in various stages of caseation and are surrounded by fibroblasts. In all the smaller tubercles the formation of fibroblasts at the periphery is well marked. Apart from the tubercular foci there is general fibroid degeneration, and atrophy of the liver cells. Bacilli are abundant in all the lesions. In the older tubercles there is a tendency for the bacilli to become longer.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Most of the tissue is replaced by large caseous masses in which bacilli are abundant.

SUMMARY.

Progressive tuberculosis ; very advanced caseation ; abundant multiplication of bacilli.

(2.)

VIRUS—P II.

Animal Inoculated.—Fowl 162.

Fowl 162 was inoculated subcutaneously with 10 mg. of culture of P II (a porcine virus with avian characters), and died 61 days afterwards.

Lungs and Kidneys.

No lesions or bacilli have been found.

Liver.

The tissue is crowded with discrete tubercles. Most of them have a broad fibrous periphery ; internal to this is a zone of nuclear aggregations, too imperfectly defined to be termed giant cells ; the central areas are completely caseous. Bacilli are numerous, both in the caseous portions and in the peripheral zones, but have not grown into rosettes.

Spleen.

The greater portion of the tissue is replaced by tubercles, in an advanced state of caseation, which resemble those found in the liver. Bacilli are more abundant than in the liver, and have grown into dense clusters and circular colonies.

SUMMARY.

Advanced caseous tubercles of the liver and spleen, with very large numbers of bacilli. The lungs seem to have escaped infection.

(3.)

VIRUS—P XXI.

Animal Inoculated.—Fowl 148.

Fowl 148 was inoculated subcutaneously with 10 mg. of culture of P XXI (a porcine virus with avian characters), and died 71 days afterwards.

Lungs.

A few minute, slightly necrotic foci are present and contain a few tubercle bacilli.

Liver.

The tissue is thickly scattered with small tubercles which have a well marked periphery of fibroblasts ; internal to this is a zone of imperfectly formed giant cells ; the centres of most of the tubercles are caseous. Bacilli are present in the tubercles but occur singly ; they never form groups or rosettes ; on the whole they are short (1 μ or less to 2 μ).

Kidneys.

There are a few small foci which are probably minute tubercles and have been overrun with lymphocytes. No bacilli have been found.

Spleen.

The tissue is riddled with caseous tubercles which are generally bounded by an abundant layer of fibroblasts. Bordering on the caseous areas there are irregular masses of imperfectly defined cell nuclei suggestive of giant cells. Bacilli are much more numerous than in the liver, and have often grown into small groups.

Local Lesion.

The local lesion is highly necrotic, but in many situations the caseous material is marked off into tubercles with boundary zones similar to those found in the liver and spleen. Bacilli occur in small groups but do not form dense colonies.

SUMMARY.

Advanced caseous tubercles of the liver and spleen.

(4.)

VIRUS—A VII.

Animal Inoculated.—Fowl 220.

Fowl 220 was inoculated subcutaneously with 10 mg. of culture of A VII, and died 47 days afterwards.

Lungs.

No lesions or bacilli have been found.

Liver.

The liver contains many tubercles which are completely caseous except at their periphery, where they contain many fibroblasts. Amongst these there are a few cells which react to Pappenheim's stain. At the periphery of the lesions oxyphil leucocytes are scanty and only a few are found in the interior; but surrounding some of the blood-vessels these cells are found in large groups. Bacilli occur in the caseous parts of the tubercles in moderate numbers.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The greater part of the tissue is replaced by tubercles in an advanced stage of caseation. These tubercles are packed closely together and are often confluent. Bacilli are scanty.

Local Lesion.

The local lesion shows a collection of caseating tubercles embedded in the underlying muscle. The tubercles contain a large amount of fibrous elements at their periphery and a few plasma cells. Oxyphil leucocytes are scattered about in small numbers but do not form large groups. Bacilli are fairly numerous in the lesions.

SUMMARY.

Advanced caseous degeneration of the spleen; many caseous tubercles in the liver; lungs normal.

(*d*) INTRAPERITONEAL INOCULATIONS IN FOWLS.

(1.)

VIRUS—P II.

Animal Inoculated.—Fowl 164.

Fowl 164 was inoculated intraperitoneally with 10 mg. of culture of P II (a porcine virus with avian characters), and died 61 days afterwards.

Lungs and Kidneys.

No lesions or bacilli have been found.

Liver.

The liver contains masses of bacilli, which form groups of colonies. Each group is the centre of a tubercle. The tubercles are partially caseous, and the larger of them have an incomplete zone of fibroblasts at their periphery; but they appear to be progressive, and none of them are completely enclosed by fibrous tissue.

Spleen.

The spleen is in the same condition as the liver, but bacilli are even more numerous and the tubercles are more closely packed together. There is less tendency to the formation of a peripheral zone of fibroblasts. There are many aggregations of nuclei in the tubercles, but no clearly defined giant cells.

SUMMARY.

Acute infection of the liver and spleen, with advanced caseation and enormous numbers of bacilli. The lungs and kidneys appear to have escaped infection.

(2.)

VIRUS—A IV.

Animal Inoculated.—Fowl 298.

Fowl 298 was inoculated intraperitoneally with 20 mg. of culture of A IV, and died 81 days afterwards.

Lungs.

There are a few small, doubtful foci, but no bacilli have been found.

Liver.

Small lesions are present, and are probably due to the action of the tubercle bacillus, but no bacilli have been found.

Kidneys and Spleen.

No lesions or bacilli have been found.

SUMMARY.

Very slight evidence of infection. No bacilli found.

(e) INOCULATION OF A FOWL WITH DEAD BACILLI.

VIRUS—A X.

Animal Inoculated—Fowl 392.

Fowl 392 was inoculated intravenously with 50 mg. of dead bacilli of A X, and died 46 days afterwards.

Lungs.

There are a few slight lesions, consisting of partially broken down cells infiltrated with leucocytes and lymphocytes. In these situations one or two fragments of bacilli have been found.

Liver.

Throughout the tissue there are numerous small groups of bacilli, many of the bacilli being fragmentary. In the situations where they occur, the bacilli have caused partial disintegration of the parenchymatous cells, together with some marginal infiltration of leucocytes and lymphocytes.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Throughout the tissue there are fairly large groups of bacilli and fragments of bacilli, which have produced areas of partial caseation. These patches are surrounded by an area of leucocytes and lymphocytes.

SUMMARY.

It appears that dead bacilli, like living bacilli, exercise a more toxic effect upon the liver and spleen than upon other organs.

(e) EXPERIMENTS ON PARROTS.

(1.)

VIRUS—A VI.

Animal Fed.—Parrot 4.

Parrot 4 was fed with 1 mg. of culture of A VI, and died 76 days afterwards.

Lungs.

There are a few large, centrally caseous lesions, and many smaller foci which are non-caseous or only slightly caseous. In all these situations bacilli are numerous; they also occur in small groups in many places where there is no tissue change.

Liver.

The liver contains many caseous tubercles. Surrounding the caseous areas there is an irregular zone of nuclei which is more or less continuous, and is not marked off into separate giant cells. The periphery of the lesions is well defined, and contains a layer of fibroblasts. Tubercle bacilli, though not very numerous, are present both in the caseous material and in the cellular zones of the tubercles.

Kidneys.

The kidneys contain breaking down lesions in which bacilli are abundant.

Spleen.

There are many caseous patches and some giant cells. Bacilli are scanty.

SUMMARY.

Disseminated, caseous lesions. Bacilli numerous in lungs and kidneys; scanty in liver and spleen.

(2.)

VIRUS—A VI.

Animal Inoculated.—Parrot 2.

Parrot 2 received an intramuscular inoculation with 10 mg. of culture of A VI, and died 95 days afterwards.

Lungs.

There are small lesions which contain swollen and slightly caseous epithelium and endothelium, and are infiltrated by lymphocytes and leucocytes. In these situations bacilli are numerous. A few isolated bacilli also occur apart from these lesions.

Liver.

There are a few small lesions which are similar in structure to those in the lungs, but no bacilli have been found in them.

Kidneys.

No lesions or bacilli have been found.

Spleen.

No lesions or bacilli have been found.

SUMMARY.

Slight lesions containing fairly numerous bacilli in the lungs; small lesions but no bacilli in the liver; no sign of infection in kidneys or spleen.

(3.)

VIRUS—A X.

Animal Inoculated.—Parrot 20.

Parrot 20 was inoculated intravenously with ·01 mg. of culture of A X, and died 113 days afterwards.

Lungs.

There are a few minute foci showing a tendency to giant cell formation. Bacilli are rare.

Liver.

Small lesions, similar to those found in the lungs, are present and occasionally contain bacilli.

Kidneys.

One lesion has been found. It consists of a group of giant cells surrounded by an infiltration composed mainly of plasma cells. The giant cells contain large numbers of bacilli.

Spleen

There are many fairly large patches of caseation and very numerous giant cells. Bacilli are abundant throughout the specimen and have grown into typical rosettes.

SUMMARY.

The spleen is severely affected and contains very numerous bacilli. The lungs, liver and kidneys are only slightly affected.

General Results.

After intravenous inoculation in fowls, severe and rapidly fatal infection is produced. The brunt of the disease falls on the liver and spleen; and in these organs there is an extremely copious multiplication of bacilli. The lungs and kidneys are much less susceptible. Many bacilli lodge in the lungs, and there multiply into colonies; but they produce very little tissue damage, much less than has been found in several cases after intravenous inoculation with highly virulent mammalian bacilli. In the kidneys also, bacilli may be detained, though in much smaller numbers than in the lungs; when detained, they frequently grow into small colonies but produce very little tissue damage.

After feeding in fowls, severe disease may be produced; but it is slower in development than after intravenous inoculation, and is associated with caseous tubercles surrounded by giant cells, or multinucleate cell masses, and a fibrous periphery. The incidence of the disease on different organs is the same as after intravenous inoculation, the liver and spleen being most severely affected.

After subcutaneous inoculation in fowls, the general results are similar to those obtained by feeding; but, in addition to the liver and spleen, the lungs may exhibit caseous nodules.

Intraperitoneal inoculation in fowls may produce similar effects to those of intravenous inoculation in the liver and spleen, but seems to be less certain in its results.

Dead bacilli, intravenously inoculated, exert a toxic effect upon the fowl, and produce more damage in the liver and spleen than in other organs.

The specimens of parrots examined show that these birds are susceptible to the avian bacillus, administered intravenously, by feeding, or by intramuscular inoculation, and that caseous lesions are readily produced.

II.—LESIONS IN CALVES.

(*a*) INTRAVENOUS INOCULATIONS.

(1.)

VIRUS—A VIII.

Animal Inoculated.—Calf 524.

Calf 524 was inoculated intravenously with 200 mg. of culture of A VIII, and died 30 days afterwards.

Lungs.

There is general congestion of the tissue and there are many necrotic patches filled with broken down leucocytes and other cells. These areas have not the appearance of tubercles, and though breaking down, do not contain a caseous deposit. Bacilli are very abundant throughout the specimen and have often grown into small solid balls about the size of a red corpuscle and sometimes about twice this size; rosette formations are also occasionally observed. The bacilli are quite as numerous in the areas showing little or no tissue change as in the necrotic lesions. Oxyphil leucocytes are very abundant within and near the breaking down foci; occasionally they contain bacilli, but not frequently. Cells reacting to Pappenheim's stain are scanty and do not contain bacilli. Throughout the tissue there are small deposits of fibrin, much of which appears to be of recent formation.

Liver.

The entire tissue is swarming with bacilli, which have grown everywhere into solid colonies and rosettes. The liver parenchyma has not been completely obliterated, even in the densest areas of bacterial invasion, but in these situations the cells stain faintly and the nuclei are distorted. Many islands and strands of intact liver cells survive, though permeated by considerable numbers of bacilli. Multinuclear leucocytes are rare, but small lymphocytes are fairly common in all parts of the tissue. No definite plasma cells have been found. Deposits of fibrin are numerous and occur where there are large collections of bacilli.

Kidneys.

There are no definite lesions, but bacilli are numerous in all parts of the specimen. They occur within glomeruli as well as in the interstitial tissue. In several places they form small solid clumps. Bacilli, though numerous, are much less abundant than in the liver ; they do not form large masses conspicuous with a low power.

Spleen.

Bacilli form dense masses in all parts of the specimen and have grown into solid colonies and rosettes. There are a few patches of slight necrosis, but the amount of tissue alteration is very small on the whole, and in most areas the presence of large numbers of bacilli does not seem to have produced any modification in the tissue.

Popliteal Gland.

Bacilli are freely disseminated throughout the gland, and have often grown into solid colonies. With the exception of a few minute patches of commencing necrosis, they have produced no appreciable alteration in the tissue cells. Leucocytes are not numerous ; they contain bacilli occasionally but only rarely. No bacilli have been found within large lymphocytes. Small deposits of fibrin are abundant and are associated with bacilli.

SUMMARY.

General dissemination with enormous multiplication of bacilli, but very little tissue destruction, and no formation of tubercles. There is well marked fibrin formation.

(2.)

VIRUS—A VIII.

Animal Inoculated.—Calf 544.

Calf 544 was inoculated intravenously with 10 mg. of culture of A VIII, and died 33 days afterwards.

Lungs.

The condition of the lungs is in every respect identical with that found in Calf 524.

Liver.

The general condition is the same as in Calf 524, the only differences noted being that (1) bacilli, though extremely numerous, are less abundant than in the animal inoculated with the larger dose, and (2) oxyphil leucocytes are more numerous and more often contain bacilli.

Kidneys.

There are no definite lesions, with the exception of small collections of cells consisting chiefly of lymphocytes and plasma cells. Bacilli occur here and there in all parts of the specimen and occasionally form a clump, but are less numerous than in the kidney of Calf 524.

Spleen.

There are more patches of definite caseation than in the spleen of Calf 524. Bacilli, though very plentiful, are rather less numerous than in the latter animal.

Portal Gland.

The gland contains enormous number of bacilli which have grown into solid colonies and rosettes. With the exception of one moderately large caseous patch, there is very little tissue destruction. Leucocytes occur in the neighbourhood of the caseous patch but are not numerous elsewhere. Some leucocytes have been found to contain bacilli. Several giant cells are found. There are a few deposits of fibrin.

SUMMARY.

With the exception of minor differences the condition is identical with that of Calf 524

(3.)

VIRUS—A X.

Animal Inoculated.—Calf 546.

Calf 546 was inoculated intravenously with 50 mg. of culture of A X, and died 32 days afterwards.

Lungs.

The tissue is highly congested, but there are no patches of complete solidification. There are no areas of necrosis or caseation, nor are there any lesions suggestive of tubercle formation. Bacilli are present throughout the tissue in large numbers, but, though evidently multiplying, have not grown into compact colonies. Bacilli sometimes occur within blood-vessels.

Liver.

Except for the presence of more lymphocytes and leucocytes than usual in the capillaries, the greater part of the tissue appears normal. There are, however, several foci where these wandering cells are collected in large numbers and the parenchymatous cells stain badly and are partially obliterated. These lesions are not sufficiently well defined to be described as tubercles. Bacilli are present in all parts of the tissue, but do not form dense groups or colonies ; they are most numerous in the lesions referred to.

Kidneys.

There are patches of infiltration with lymphocytes, leucocytes, and plasma cells, but no well defined lesions. Bacilli are present in the interstitial tissue but are scanty.

Popliteal Gland.

There are several small foci showing a slight tendency to necrotic change but, except for these, the tissue appears normal. Bacilli are distributed in all parts of the gland, including these lesions, but are not numerous and have not grown into groups or colonies.

SUMMARY.

There is a general dissemination of bacilli in moderate numbers but very little tissue destruction, and no formation of typical tubercles.

(4.)

VIRUS—A X.

Animal Inoculated.—Calf 558.

Calf 558 was inoculated intravenously with 5 cc. of an emulsion of the liver of Calf 562 (infected with the virus A X), and died 45 days afterwards.

Lungs.

The tissue is in the same general condition as the lungs of Calf 524, and the bacilli are quite as numerous ; but, in the present specimen the general congestion is less severe and the patches of nuclear débris are smaller.

Liver.

In the liver, bacilli, though generally distributed, are relatively scanty, a condition which is in marked contrast to the enormous number of bacilli present in the lungs. The histological changes are not very pronounced but are identical with those found in the liver of the preceding animal, Calf 546.

Kidneys.

Identical with the kidneys of Calf 546.

Spleen.

There are numerous imperfectly defined areas which are slightly caseous and contain many leucocytes and the remains of these cells. In these lesions bacilli are numerous and are commencing to form colonies. Apart from these lesions, bacilli are generally distributed, and, though less plentiful on the whole, have occasionally grown into colonies.

Mediastinal Gland.

The tissue is swarming with bacilli, and though the amount of tissue destruction is very slight in many places, there are also fairly large tracts of caseo-necrotic material

SUMMARY.

General dissemination ; abundant multiplication of bacilli in the lungs and mediastinal gland ; bacilli much less numerous in the liver ; a tendency to caseation in the mediastinal gland and spleen.

(5.)

VIRUS—A X.

Animal Inoculated.—Calf 560.

Calf 560 was inoculated intravenously with 9 cc. of an emulsion from the liver of Calf 554 (infected with the virus A X), and died 40 days afterwards.

Lungs.

The specimen of lung shows a generalised and nearly complete consolidation. The condition is unlike that produced in tubercular pneumonia and is characterised by the presence of extremely large numbers of multinuclear leucocytes, the occlusion of the alveoli being mainly due to these cells. Bacilli are abundant all over the tissue, and have often grown into solid colonies and rosettes. Bacilli are found in a moderate number of the leucocytes, but the majority of these cells seem indifferent to the bacilli.

Liver.

The condition is the same as in the liver of Calf 544.

Kidneys.

Identical with the kidneys of Calf 544.

Spleen.

Bacilli are plentiful all over the specimen and here and there have grown into colonies. There is very little indication of caseation or tissue destruction, but occasionally there is a tendency to cell fusions and partial formation of giant cells.

Popliteal Gland.

Numerous bacilli are distributed about the gland, but have not produced caseation or other definite lesions.

SUMMARY.

General dissemination and multiplication of bacilli ; no lesions typical of tuberculosis.

(6.)

VIRUS—A X.

Animal Inoculated—Calf 564.

Calf 564 was inoculated intravenously with 9 cc. of an emulsion derived from the liver of Calf 560, and died 45 days afterwards.

Lungs.

The lungs are highly congested, but there is no breaking down of tissue and no tubercle formation. Bacilli are swarming all over the specimen, and are very frequent in the blood-vessels ; they have grown into small colonies but do not form definite rosettes.

Liver.

Bacilli are distributed abundantly throughout the tissue and have frequently grown into small colonies. They have produced lesions characterised by a partial breaking down of the liver cells and an invasion of lymphocytes and leucocytes. The interior of liver cells appears to be the most favourable situation for the growth of bacilli. Many bacilli are found within blood-vessels.

Kidneys.

Bacilli are numerous, though less abundant than in the lungs or liver, and are associated with a cellular infiltration consisting chiefly of lymphocytes and plasma cells. There is no breaking down of tissue. Some bacilli are present in the blood-vessels.

Spleen.

Bacilli are very abundant and have grown into colonies. There is very little tissue destruction, but a few slightly caseous patches have been observed.

Mediastinal Gland.

The gland is swarming with bacilli, which have frequently grown into colonies. There are many slightly caseous patches and several giant cells.

SUMMARY.

General dissemination and enormous multiplication of bacilli. Very little tissue destruction

(7.)

VIRUS—A X.

Animal Inoculated.—Calf 566.

Calf 566 was inoculated intravenously with 5 cc. of an emulsion of the tissues of Calf 558, and was killed, when ill, 43 days afterwards.

Lungs.

The specimen is solid and is packed with leucocytes. No tubercle bacilli are present.

Liver, Kidneys, and Spleen.

Neither tubercle bacilli nor lesions suggestive of tuberculosis have been found.

Cervical Gland.

The gland is extensively caseous and contains large numbers of bacilli, but these have not grown into colonies or rosettes. Some of the bacilli are contained within leucocytes.

SUMMARY.

No evidence of bacilli except in the cervical gland; death apparently due to pneumonia.

(8.)

VIRUS—A X.

Animal Inoculated—Calf 570.

Calf 570 was inoculated intravenously with 5 cc. of an emulsion of the liver of Calf 564, and died 31 days afterwards.

Lungs and Liver.

The lungs and liver are in the same condition as the corresponding organs of Calf 564.

Kidneys.

Bacilli are very scanty. There are a few patches of interstitial cellular infiltration.

Spleen.

Similar to the spleen of Calf 564.

SUMMARY.

Similar to Calf 564, but fewer bacilli in the kidneys.

(*b*) SUBCUTANEOUS INOCULATIONS.

(1.)

VIRUS—P III.

Animal Inoculated.—Calf 318.

Calf 318 was inoculated subcutaneously with 50 mg. of culture of P III (a porcine virus with avian characters), and was killed 95 days afterwards.

Lungs, Liver, Kidneys, and Cœliac Gland.

No lesions or bacilli have been found.

Mesenteric Gland.

There are one or two minute caseous foci. One bacillus has been found.

Prescapular Gland.

There are very extensive caseo-calcareous lesions. Surrounding these there is a broad layer of fibroblasts. Some imperfectly formed giant cells are present. Tubercle bacilli are scanty.

SUMMARY.

The infection appears to have been almost completely limited to the nearest glands.

(2.)

VIRUS—P XXI.

Animal Inoculated.—Calf 412.

Calf 412 was inoculated subcutaneously with 50 mg. of culture of P XXI (a porcine virus with avian characters), and was killed 99 days afterwards.

Lungs.

Two pieces of tissue have been examined but no tubercles or tubercle bacilli have been found.

Liver.

In one specimen no lesions or bacilli are present. In another specimen there are three or four small foci which are in more or less advanced stages of caseation and are surrounded by fibroblasts; in two of these, one or two bacilli are present.

Kidneys.

In the two specimens examined no lesions or bacilli have been found.

Portal Gland.

There are numerous caseous areas. In some of these tubercle bacilli are scanty; in others they are abundant. Giant cells are numerous.

SUMMARY.

No lesions have been found in the lungs or kidneys. The liver contains some small tubercles, of a chronic type, containing very few bacilli. In a portal gland there are several caseous areas, and in some of these bacilli are numerous.

(3.)

VIRUS—A II.

Animal Inoculated.—Calf 432.

Calf 432 was inoculated subcutaneously with 50 mg. of culture of A II, and was killed 101 days afterwards.

Lungs.

In one specimen no lesions or bacilli are present. In a second specimen there is a caseous focus about the area of the microscopic field (low power) in which one bacillus has been found.

Liver and Kidneys.

There are some small, doubtful foci in which no bacilli have been found.

Prescapular Gland.

Most of the normal tissue is replaced by caseous areas which show a tendency to calcification, and are surrounded by broad fibrous zones where the transition of plasma cells into fibroblasts is well marked. Bacilli are present in the lesions in small numbers.

SUMMARY.

There has been some dissemination, but the lesions contain few bacilli and are in a markedly retrogressive condition.

General Results.

I. INTRAVENOUS INOCULATIONS.

This method of inoculation produces rapidly fatal infection in young calves. It is associated with general dissemination and very abundant multiplication of the bacilli; but there is relatively little tissue destruction and there is an absence of tubercle formation: These results offer an interesting resemblance to the condition found in young calves which have rapidly succumbed to the intravenous inoculation of strains of mammalian bacilli possessing low virulence for the bovine when inoculated subcutaneously. But the avian bacilli multiply even more profusely, and often grow into the solid colonies and rosettes which are characteristic of this bacillus. In one exceptional case, Calf 566, no tubercle bacilli were found in most of the tissues examined; death appears to have been due to pneumonia.

Apart from other toxic effects which may have been associated with the profuse growth and dissemination of the avian bacilli, the anatomical condition of the lungs seems to have been sufficient to account for death. To the naked eye the specimens presented extreme oedema with patches of complete consolidation, some in the red and some in the grey stage. These grey patches were sometimes surrounded by areas in the red stage, but, as my microscopic examinations show, they were not caseous tubercles.

Though the total amount of gross tissue destruction was small, as compared with the acute disease produced by highly virulent mammalian bacilli after subcutaneous inoculation of a large dose, microscopic examination showed, particularly in the liver, that the tissue cells were not left in a normal condition. The livers of Calves 524 and 544, for example, when stained appropriately, showed extensive damage to the parenchymatous cells.

II. SUBCUTANEOUS INOCULATIONS.

The subcutaneous inoculation of 50 mg. of culture has failed to produce more than a mild degree of general dissemination, and in some cases the disease appears to have been almost limited to the nearest glands. The disseminated lesions which have been found are usually of a definitely retrogressive character, and bacilli, when present in them, are rarely numerous. Caseation and giant cell formation are not uncommon, and the general structure of some of the lesions is indistinguishable from that of chronic tubercles produced by mammalian bacilli.

III.—LESIONS IN RABBITS.

(a) INTRAVENOUS INOCULATIONS.

(1.)

VIRUS—P II.

Animal Inoculated—Rabbit 1538.

Rabbit 1538 was inoculated intravenously with 50 mg. of culture of P II (a porcine virus with avian characters), heated at 70° for 30 minutes, and was killed 36 days afterwards.

Lungs.

The tissue is considerably congested, but there are no lesions suggestive of tuberculosis, and only two tubercle bacilli have been found.

Liver.

There are a few giant cells and other minute, suspicious foci. Two tubercle bacilli have been found.

Kidneys.

Neither lesions nor bacilli have been found.

Spleen.

Single bacilli have been found in several places, but the total number is small ; they have produced very little tissue disturbance.

SUMMARY.

The bacilli have nearly all disappeared ; no definite lesions typical of tuberculosis have been produced.

(2.)

VIRUS—P II.

Animal Inoculated.—Rabbit 1539.

Rabbit 1539 was inoculated intravenously with 50 mg. of a broth culture, killed by heating at 100°, of P II (a porcine virus with avian characters), and was killed 36 days afterwards.

Lungs.

There is some general thickening of the alveolar walls and several small patches of consolidation are present which are slightly caseous in the centre. These lesions are infiltrated with leucocytes and show a peripheral zone of lymphocytes and plasma cells ; they present the general appearance of chronic lesions produced by mammalian bacilli. Bacilli are scattered about in these lesions in small numbers and also occur singly in other parts of the specimen. No bacilli have been found within leucocytes, nor within blood-vessels.

Liver.

A few bacilli are present and have produced minute lesions, consisting of partial swelling up and disintegration of the liver cells and endothelial cells in contact with the bacilli, together with the accession of two or three lymphocytes and leucocytes. No bacilli have been found within blood-vessels.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Bacilli are scattered about the specimen in small numbers. Many of them are not associated with any lesions, but a few are within, or near to, small multinucleate foci which are hardly large enough to be called giant cells.

SUMMARY.

Numerous small, slightly caseous lesions in the lungs, indistinguishable from lesions produced by mammalian bacilli. Very little tissue change in liver or spleen.

(3.)

VIRUS—P II.

Animal Inoculated—Rabbit 1540.

Rabbit 1540 was inoculated intravenously with 10 mg. of culture of P II (a porcine virus with avian characters), killed by steaming at 100° for 30 minutes, and was killed 36 days afterwards.

Lungs.

The tissue is congested and there are a few suspicious foci, but these are not definite enough to be called tubercles. No bacilli have been found.

Liver.

There are no lesions large enough to be called tubercles, but single giant cells occur frequently ; in a few of these, one or two bacilli are present.

Kidneys.

Neither lesions nor bacilli have been found.

Spleen.

A few minute lesions. Bacilli very rare.

SUMMARY.

The bacilli have nearly all disappeared, and have only produced small foci of irritation, not definite enough to be called tubercles.

(4.)

VIRUS—P XXI.

Animal Inoculated.—Rabbit 880.

Rabbit 880 was inoculated intravenously with ·001 mg. of culture of P XXI (a porcine virus with avian characters), and was killed 140 days afterwards.

Lungs.

The lungs contain fairly large nodules, which are in an advanced stage of caseation and are surrounded by a layer of plasma cells and lymphocytes. Internal to this layer there is a zone of epithelioid cells resembling embryonic alveolar epithelium, and amongst these there is a sprinkling of oxyphil leucocytes. In the caseous material bacilli are sometimes abundant, and are often long and irregularly stained, but in the peripheral portions of the nodules bacilli are scanty.

Liver.

Both within and between the lobules, foci are present which are in various stages of caseation, and in some of these a few tubercle bacilli are present. Plasma cells are very conspicuous both at the periphery of these foci and in the interlobular tissue.

Kidneys.

There are several large caseous tubercles, and also smaller lesions, which are commencing to caseate. At the periphery of the lesions there are dense masses of plasma cells, and in their interior are numerous oxyphil leucocytes. Tubercle bacilli are fairly numerous in the caseous areas, and are frequently long and curved ; in the cellular portions of the lesions they are much less frequent and are usually short and straight.

Spleen.

The spleen contains fairly large caseous lesions. Both in these situations and throughout the rest of the tissue bacilli are scattered in moderately large numbers. Leucocytes are very abundant.

SUMMARY.

Disseminated tuberculosis of a chronic type.

(5.)

VIRUS—P XXVII.

Animal Inoculated.—Rabbit 775.

Rabbit 775 was inoculated intravenously with 1 mg. of culture of P XXVII (a porcine virus with avian characters), and died 25 days afterwards.

Lungs.

The tissue is in a semi-solid condition, consisting of innumerable consolidated foci interspersed between greatly thickened alveolar walls. The general condition resembles, in many respects, that produced by intravenous inoculation with virulent mammalian bacilli, but under the latter condition there would have been more complete consolidation and more extensive caseation. Bacilli have not grown into colonies but are numerous in all situations and are frequent within blood-vessels, where they are generally attached to, or within, detached endothelial cells. The bacilli have not produced rosette formations.

Liver.

The tissue is swarming with bacilli, which have produced the characteristic rosette formations. A special feature of the section is the large number of multinucleated cells which contain a broad peripheral ring of bacilli surrounding a centrally placed mass of nuclei.

Kidneys.

There are no noticeable lesions, and bacilli are scanty, but small groups of them are present within a few of the glomeruli.

Spleen.

The tissue is swarming with bacilli which have grown everywhere into colonies and rosettes. Giant cells with central aggregations of nuclei are numerous and conspicuous. Leucocytes are not very numerous ; a few of them contain small clumps of bacilli.

Mesenteric Gland.

Bacilli are distributed throughout the gland and in some places have grown into rosettes. There are patches of slight caseation and a few giant cells.

SUMMARY.

Typical avian tuberculosis in the rabbit.

(6.)

VIRUS—A X.

Animal Inoculated.—Rabbit 1791.

Rabbit 1791 was inoculated intravenously with 1 mg. of culture of A X, and died 18 days afterwards.

Lungs.

The tissue is greatly congested and there is an abundant infiltration, in which lymphocytes greatly preponderate over leucocytes. There are no definitely caseous or tubercular lesions, but bacilli are scattered about all parts of the tissue, and in many places have grown into small groups. No bacilli have been found within leucocytes.

Liver.

Bacilli are abundant all over the specimen, and have formed typical rosettes. The bacilli show a marked tendency to grow within the parenchymatous cells, and there is a general infiltration with lymphocytes and leucocytes. A small proportion of the leucocytes are phagocytic.

Kidneys.

Bacilli are distributed singly and in small groups throughout the specimen. There is an infiltration of lymphocytes and a smaller number of leucocytes, but these cells are not closely associated with the bacilli.

Spleen.

Bacilli are very abundant; they have grown into rosettes and colonies, and have produced diffuse areas of partial necrosis. There is only slight evidence of leucocytic phagocytosis.

Bronchial Gland.

Bacilli are scattered throughout the gland in fairly large numbers and in some places have grown into colonies and rosettes. There is very little tissue destruction.

SUMMARY.

General dissemination and abundant multiplication of bacilli, with relatively little tissue destruction.

(*b*) FEEDING EXPERIMENTS.

(1.)

VIRUS—P XXI.

Animal Fed.—Rabbit 1092.

Rabbit 1092 was fed once with 20 mg. of culture of P XXI (a porcine virus with avian characters), and died 195 days afterwards.

Lungs.

There are a few small foci which consist of aggregations of leucocytes and lymphocytes and contain an occasional tubercle bacillus.

Liver.

A few caseous foci, surrounded by a broad zone of fibroblasts, are present. There are also several rather smaller lesions which are only partially caseous, are infiltrated with lymphocytes and leucocytes, and exhibit no peripheral zone of fibroblasts. Bacilli are present in some of the lesions but are rare.

Kidneys.

A few caseous lesions are present. These are surrounded by large groups of lymphocytes and leucocytes and contain fairly numerous bacilli.

SUMMARY.

The bacilli have become disseminated in small numbers and have produced lesions which are not of an acute type.

(2.)

VIRUS—P XLIV.

Animal Fed.—Rabbit 1180.

Rabbit 1180 was fed once with 10 mg. of culture of P XLIV (a porcine virus with avian characters), and died 323 days afterwards.

Lungs.

The lungs contain many small lesions which are centrally caseous and resemble lesions found in mild cases of infection with mammalian bacilli. Giant cells are found at the periphery of some of the lesions. Bacilli are present but scanty.

Liver.

There are several small, slightly caseous tubercles, but no bacilli have been found in them.

Kidneys.

In the specimen examined there is a circumscribed, caseous tubercle. Several bacilli are present in the caseous material, and a few also occur in the cellular periphery.

SUMMARY.

Some dissemination has occurred, and chronic lesions of a mild type have been produced. Bacilli are scanty. The lesions cannot be distinguished from lesions produced by mammalian bacilli.

(3.)

VIRUSES—A I and A VII.

Animals Fed.—Rabbits 1090 and 1282.

Rabbit 1090 was fed with 1 mg. of A I, and was killed 163 days afterwards. Rabbit 1282 was fed with 10 mg. of A VII, and was killed 91 days afterwards.

There is very little evidence of infection in these animals. In Rabbit 1090 neither lesions nor bacilli have been found in the specimens of lungs, liver, kidneys, and a mesenteric gland. In Rabbit 1282 no evidence of infection has been found in the lungs, kidneys, or spleen; one bacillus has been found in the liver, and a few bacilli are present in the walls of the small intestine.

SUMMARY.

Almost complete resistance to infection.

(c) SUBCUTANEOUS INOCULATIONS.

(1.)

VIRUS—P II.

Animal Inoculated—Rabbit 326.

Rabbit 326 was inoculated subcutaneously with 5 mg. of culture of P II (a porcine virus with avian characters), and died 69 days afterwards.

Lungs.

The lungs are congested and contain numerous small consolidated foci which have no definite boundary zone but are filled with leucocytes and lymphocytes. They are not surrounded by fibroblasts, nor do they contain any giant cells. Oxyphil leucocytes are numerous not only in these areas, but also in the rest of the tissue. A few of the foci are slightly caseous in the centre. No fibrin is present, nor have any typical plasma cells been found.

Tubercle bacilli are present in the areas referred to but are rare, and only occur singly.

Liver.

As in the lungs, small lesions are distributed abundantly throughout the tissue and are characterised by the presence of numerous leucocytes and lymphocytes and the absence of fibroblasts and giant cells. Some of the foci are centrally caseous, and in some the capillary walls have broken down and there are small extravasations of blood corpuscles. In some of these last situations a scanty deposit of early fibrin is found. Tubercle bacilli are present in the lesions but are rare.

Kidneys.

In the kidneys lesions are fairly numerous, and some of them are extensively caseous. The cells infiltrating and forming the boundary layer of these areas are mainly lymphocytes, though a relatively small number of leucocytes is also present. Bacilli are more numerous than in either the lungs or liver. They are loosely scattered in all parts of the lesions, both in the caseous areas and in the cellular periphery; they have not grown into clumps.

Mesenteric Gland.

The gland contains extensive, ramifying, semi-caseous tracts which are filled with the nuclear fragments of leucocytes and other cells. There is an absence of new connective tissue formation and of giant cells. The number of normal oxyphil leucocytes (*i.e.*, with well-marked granular protoplasm) is not large. Many of the blood-vessels contain deposits of fibrin, but apart from these situations there is no evidence of fibrin formation. The gland is swarming with bacilli, which, in many situations, have grown into clusters of small colonies. Many of these colonies tend to assume a rosette shape. The great majority of the bacilli are very short, straight, and uniformly stained.

SUMMARY.

General dissemination; in the lungs, liver, and kidneys there is a vigorous reaction on the part of the leucocytes and lymphocytes, and bacilli are not abundant; in the mesenteric gland bacilli are very abundant, and there is extensive tissue destruction. The absence of giant cells and of new connective tissue formation is noteworthy.

(2.)

VIRUS—P II.

Animal Inoculated.—Rabbit 1541.

Rabbit 1541 was inoculated subcutaneously with 250 mg. of dead broth culture of P II (a porcine virus with avian characters), and was killed 36 days afterwards.

Lungs.

One small group of bacilli has been found. There are no tubercles, nor does the tissue afford any indication that bacilli have reached the lungs in large numbers.

Liver.

A few bacilli have been found in a blood-vessel. There are also a few bacilli which occur in small foci consisting of partially disintegrated liver cells and endothelial cells together with a few lymphocytes and leucocytes.

Kidneys and Spleen.

No lesions or bacilli have been found.

Local Lesion.

The centre of the local lesion is caseous and contains large numbers of bacilli. At the periphery, where bacilli rarely occur, there is a broad zone of fibroblasts, lymphocytes, and plasma cells, separating the lesion from the subjacent muscle. Leucocytes are abundant, particularly in the intermediate area, and one or two have been found containing bacilli; in the caseous material also a few of the leucocytes present contain bacilli.

SUMMARY.

There is slight evidence of dissemination, but the great bulk of the inoculated material does not appear to have escaped beyond the site of inoculation.

(3.)

VIRUS—P XXI.

Animal Inoculated.—Rabbit 878.

Rabbit 878 was inoculated subcutaneously with 1 mg. of culture of P XXI (a porcine virus with avian characters), and died 155 days afterwards.

Lungs.

The lungs are in a semi-solid condition, with commencing caseation. Tubercle bacilli are scanty.

Kidneys.

There are many small areas of infiltration. Bacilli are not very numerous.

Spleen.

Throughout the tissue bacilli are distributed in scanty numbers. There is no tissue destruction but there are patches of infiltration with lymphocytes and plasma cells.

SUMMARY.

Bacilli are disseminated in scanty numbers; the disease appears to have been slowly progressive in the lungs.

(4.)

VIRUS—A V.

Animal Inoculated.—Rabbit 956.

Rabbit 956 was inoculated subcutaneously with 10 mg. of culture of A V, and died 52 days afterwards.

Lungs.

There is some thickening of the alveolar walls, but no bacilli or lesions suggestive of tuberculosis have been found in the specimens examined.

Liver.

Small tubercles in an early stage of caseation, containing giant cells and not surrounded by fibroblasts, are distributed abundantly throughout the tissue. They resemble lesions produced by mammalian bacilli. Bacilli are present but are scanty; there are no rosette formations.

Kidneys.

Several small lesions are present. The larger of them are centrally caseous and are infiltrated with leucocytes and surrounded by a broad margin of lymphocytes and plasma cells. Bacilli are scanty and only occur singly.

Bronchial Gland.

The gland is extensively caseous and contains bacilli in large numbers. Only one or two suggestions of a rosette formation have been observed.

SUMMARY.

Disseminated tuberculosis, not of an acute type. Tubercles are particularly numerous in the liver. Bacilli are scanty. The lesions are indistinguishable from tubercles produced by mammalian bacilli.

(5.)

VIRUS—A V.

Animal Inoculated.—Rabbit 957.

Rabbit 957 was inoculated subcutaneously with 10 mg. of culture of A V, and was killed 168 days afterwards.

Lungs.

The tissue is congested and contains many isolated foci which are in a more or less advanced stage of caseation. These foci are all surrounded by a well marked zone of plasma cells and contain in their interior numerous oxyphil leucocytes. No giant cells have been found. Most of the foci are small and contain very few bacilli, but in one, which is much larger than the rest and is extensively necrotic, bacilli are very abundant.

Liver.

There are many small patches characterised by aggregations of lymphocytes and the presence of many imperfectly formed giant cells. In a few of these situations one or two bacilli have been found.

Kidneys.

A few suspicious patches of infiltration have been noted but no definite tubercles or tubercle bacilli.

Spleen.

No lesions or tubercle bacilli have been found.

SUMMARY.

The lungs, which are more severely affected than the other tissues examined, contain numerous caseating lesions; bacilli, though rare in most of these foci, are very abundant in one of the largest.

(*d*) INTRAPERITONEAL INOCULATIONS.

(1.)

VIRUS—P II.

Animal Inoculated.—Rabbit 292.

Rabbit 292 received an intraperitoneal inoculation of 1 mg. of culture of P II (a porcine virus with avian characters), and died 22 days afterwards.

Lungs.

The lungs are semi-solid, irregular air spaces being left between groups of consolidated alveoli. The process appears to be due to an infiltration and expansion of the alveolar walls. A few of the consolidated foci are slightly necrotic in the centre, but the majority of them show no signs of necrosis. Oxyphil leucocytes are very numerous, and the foci also contain many lymphocytes and a fairly large sprinkling of plasma cells. The lesions contain no giant cells and are not bounded by a fibrous zone. Bacilli are abundant throughout the tissue and have grown everywhere into small colonies. The bacilli are very small, many of them being less than 1 μ in length.

Liver.

In eosin and methylene blue and in Pappenheim specimens the tissue is riddled with pale areas between which a network of more darkly staining, normal parenchymatous cells has survived. The pale areas consist of disintegrated liver cells and the cells belonging to the normal capillary channels; amongst these cells there is a sprinkling of oxyphil leucocytes and an occasional plasma cell. A few of the foci are necrotic in the centre, and here nuclear fragments and oxyphil granules belonging to the leucocytes are to be found. The foci have no peripheral zone of any sort and contain no giant cells. Small groups of plasma cells are present amongst the interlobular connective tissue. All the pale areas referred to are found to contain large numbers of bacilli. The bacilli have a marked tendency to arrange themselves in the form of a rosette, with a clear central space. They are short and straight, resembling those found in the lungs. There is a scanty deposit of fibrin in many of the foci which show signs of necrosis.

Kidneys.

The tissue contains numerous small groups of bacilli, which occur sometimes in glomeruli and sometimes in the interstitial tissue. Surrounding the bacilli there are aggregations of leucocytes and lymphocytes, and in one situation, immediately beneath the capsule, there is a central area of necrosis.

Spleen.

The spleen is crowded with groups of bacilli, which show a marked tendency to assume the rosette form. In several places the bacilli have produced areas of almost complete necrosis. There is a marked leucocytosis.

Mesenteric Gland.

The gland contains large groups of bacilli which form colonies possessing the characteristic rosette appearance, and are arranged at the periphery of completely necrotic areas, in the centre of which the bacilli are fewer, more scattered, and longer. There is an aggregation of oxyphil leucocytes external to the colonies of bacilli.

SUMMARY.

General dissemination; abundant multiplication of bacilli; necrotic change in most of the organs.

(2.)

VIRUS—P II.

Animal Inoculated.—Rabbit 294.

Rabbit 294 received an intraperitoneal inoculation with 50 mg. of culture of P II (a porcine virus with avian characters), and died 13 days afterwards.

Lungs.

The alveolar walls are generally thickened and there are many small consolidated patches. These areas are only slightly necrotic; most of them are surrounded by a ring of plasma cells. Throughout the whole of the tissue oxyphil leucocytes are very abundant. There is no definite evidence of fibrinous deposit Tubercle bacilli are present in all parts of the tissue and are numerous in the consolidated foci. They are loosely scattered, and, though evidently multiplying, have not grown into rosette formations.

Liver.

Bacilli are disseminated copiously throughout the tissue and have grown everywhere into colonies with a characteristic rosette formation. There are many small deposits of fibrin, but these are not definitely associated with groups of bacilli. Plasma cells are well marked in the interstitial tissue and occur scantily within the lobules. In eosin and methylene blue specimens it is seen that the normally staining liver parenchyma has to a large extent been replaced by areas of diffuse infiltration. In these areas the parenchymatous cells have lost their eosinophil protoplasmic stain; giant cells are numerous but there is no caseation; oxyphil leucocytes occur everywhere in large numbers. The nuclei of many of the giant cells form a well marked ring, and many of the nuclei contain a large nucleolus which takes the eosin stain intensely. Comparing this specimen with the carbol-fuchsin specimen, it is found that the ring of nuclei in the giant cells generally corresponds in position to the periphery of a colony of bacilli. In the central clear space of the colonies which show a rosette formation, cell nuclei are generally absent. It seems clear from these specimens that the characteristic formation of these colonies is not due to a process of growth round a cell nucleus.

Kidneys.

A few minute groups of bacilli are present. Some of these occur within glomeruli. No histological changes have been found.

Spleen.

As in the liver, bacilli are abundant in all parts of the specimen and have grown into colonies and rosettes; there is a tendency to giant cell formation.

Mesenteric Gland.

There are large caseous areas which contain very numerous bacilli. The rosette formation of the colonies is well marked.

SUMMARY

In the liver and mesenteric glands the bacilli have multiplied very abundantly and have caused advanced tissue changes. The lungs are also severely affected, though less severely than the liver. Very few bacilli, and no tissue changes, have been found in the kidney.

(3.)

VIRUS—P XLIV.

Animal Inoculated.—Rabbit 1183.

Rabbit 1183 received an intraperitoneal inoculation with 1 mg. of culture of P XLIV (a porcine virus with avian characters), and died 37 days afterwards.

Lungs.

Many of the alveolar walls are slightly thickened and contain numerous oxyphil leucocytes. There are also many small consolidated foci which are not caseous and have not the structure of definite tubercles, but consist of irregular groups of swollen epithelial and endothelial cells infiltrated with leucocytes, lymphocytes, and a few plasma cells. Tubercle bacilli are present in moderate numbers both in these foci and in many parts of the thickened alveolar walls. No fibrin is present.

Liver.

The tissue is riddled with enormous numbers of bacilli which have grown everywhere into typical rosette formations. They have produced partial obliteration of the parenchymatous cells and many areas of slight necrosis, but have not led to the formation of tubercles.

Kidneys.

Bacilli occur in many parts of the interstitial tissue and occasionally within glomeruli. They are much less abundant than in the liver. No tubercles are present, but there is a patchy infiltration of lymphocytes and plasma cells.

Mesenteric Gland.

Bacilli are abundant and have often formed rosettes There are many areas of early caseation.

SUMMARY.

General dissemination with particularly severe incidence on the liver.

General Results.

(1) *Intravenous Inoculation.*—When dead bacilli are inoculated, even in so large a dose as 50 mg., the bacilli are rapidly eliminated. Traces of their presence may be found in small foci of irritation, more particularly in the lungs, where there may be produced small, slightly caseous lesions indistinguishable from lesions produced by mammalian bacilli. The inoculation of a very small dose of living bacilli caused disseminated tuberculosis of a chronic and relatively mild type, the lesions being discrete, circumscribed, and centrally caseous. When a larger dose, *e.g.* 1 mg., is inoculated, rapidly fatal disease is produced; the bacilli become disseminated throughout the body and grow very abundantly, forming numerous colonies and rosettes; the amount of tissue destruction is relatively slight, though severe irritant effects may be observed in the lungs; the bacilli show a marked tendency to intracellular growth, and though they lead to the formation of giant cells, tubercles of the discrete, caseous type are rare.

(2) *Feeding.*—This is a much less severe mode of infection. Disseminated lesions may be found, but they are small and of the chronic, caseous type.

(3) *Subcutaneous Inoculation.*—Disseminated caseous tubercles are frequently produced. They usually contain relatively few bacilli, but may be extensive and progressive.

(4) *Intraperitoneal Inoculation.*— This method of infection causes general dissemination and abundant multiplication of bacilli. and its effects are comparable to those produced by intravenous inoculation.

IV.—LESIONS IN MONKEYS.

(*a*) SUBCUTANEOUS INOCULATION.

VIRUS—P II.

Animal Inoculated.—Monkey 118.

Monkey 118 was inoculated subcutaneously with 10 mg. of culture of P II (a porcine virus with avian characters), and was killed 97 days afterwards.

Lungs.

One specimen is completely consolidated and almost wholly necrotic; it contains a considerable amount of fibrin, both old and recent. In another specimen, though the majority of the alveoli are still patent, the tissue as a whole is congested and there are several tubercles which are filled with masses of multinuclear leucocytes. Throughout the first specimen and in the tubercles present in the second specimen bacilli are very numerous; they have not grown into solid clumps, nor assumed a rosette formation.

Liver.

There are numerous semi-necrotic, miliary tubercles which contain fibrin and possess no demarcation zone. Some of them are crowded with multinuclear leucocytes and fragments of these cells. Bacilli are numerous in the tubercles and are distributed loosely, as in the lungs.

Kidneys.

Large, necrotic tubercles are present, and there is some fibrinous deposit. Bacilli are abundant.

Portal Gland.

Extensively caseous; bacilli abundant.

SUMMARY.

Acute disseminated tuberculosis, identical with the disease produced by virulent mammalian bacilli.*

(*b*) INTRAVENOUS INOCULATIONS.

(1.)

VIRUS—P XXI.

Animal Inoculated.—Monkey 154.

Monkey 154 was inoculated intravenously with 1 mg. of culture of P XXI (a porcine virus with avian characters), and died 34 days afterwards.

Lungs.

There is some slight thickening of the alveolar walls, and there are a few small consolidated patches which are slightly caseous in the centre and are surrounded by leucocytes and lymphocytes at their periphery. A few bacilli are present in these areas.

Liver.

There is a general infiltration with lymphocytes and leucocytes, which is particularly well marked in the interlobular tissue and surrounding the blood-vessels, both portal and hepatic. The liver parenchyma is crowded with small, irregular areas, where the liver cells stain badly and are infiltrated with lymphocytes and leucocytes. These areas are not definitely caseous and do not contain giant cells. In some of these areas tubercle bacilli are present, but only in scanty numbers. Owing to the general scarcity of the tubercle bacilli, it is difficult to say whether the condition of the liver is entirely due to these organisms.

Kidneys.

No tubercle bacilli or lesions suggestive of tuberculosis have been found.

SUMMARY.

The lungs contain a few consolidated patches in which bacilli are present in scanty numbers. The liver also contains small lesions in which bacilli are found.

(2.)

VIRUS—A I.

Animal Inoculated.—Monkey 160.

Monkey 160 was inoculated intravenously with 10 mg. of culture of A I, and died 19 days afterwards.

Lungs.

The tissue is congested and the alveolar walls are much thickened, but there are no lesions suggestive of tuberculosis. Bacilli are present, but are very scanty; they have produced no definite tissue reaction.

Liver.

The tissue consists of a network of intact liver cells enclosing areas where the parenchymatous cells are not obliterated, but have lost their protoplasmic stain and are infiltrated with lymphocytes and a smaller number of leucocytes. These areas have not the appearance of tubercles, and do not contain giant cells or fibrin. They contain bacilli in large numbers, and in a few instances the bacilli have grown into the form of a rosette. The bacilli are notably short and straight (·5 to 2 or 2·5 μ).

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* This result is exceptional; other monkeys proved resistant to subcutaneous inoculation, and did not yield material useful for microscopic examination.

Kidneys.

There is a slight degree of interstitial infiltration, but no appearance suggestive of tuberculosis. Bacilli occur, but only in twos and threes, and are very scanty.

Spleen.

Bacilli are disseminated throughout the organ in large numbers, and in several places have assumed a rosette formation; they are of the same characters as those found in the liver. There is no caseation or giant cell formation, but the presence of the bacilli appears to be associated with an excess of lymphocytes.

Inguinal Gland.

A few bacilli are present, but have produced no definite tissue change.

SUMMARY.

Bacilli have multiplied abundantly in the liver and spleen and have caused some tissue change, but have not produced tubercles. The lungs are only slightly affected and contain few bacilli.

(3.)

VIRUS—A X.

Animal Inoculated.—Monkey 188.

Monkey 188 was inoculated intravenously with 50 mg. of culture of A X, and died 18 days afterwards.

Lungs.

Bacilli are distributed throughout the tissue in fairly large numbers, and often form small clumps; bacilli are also frequently found in the blood-vessels. The alveolar walls are swollen, but there are no patches of consolidation and no signs of tissue destruction. Many of the bacilli are within epithelial or endothelial cells. Leucocytes are not greatly in excess of the normal.

Liver.

Bacilli are distributed throughout the tissue in very large numbers, and have frequently grown into rosettes. In general appearance the specimen resembles a rabbit's liver after intravenous inoculation with avian bacilli. The bacilli have grown most freely within epithelial and endothelial cells; they have produced partial disintegration of the liver cells and swelling up of the endothelial cells, but no caseation and no giant cell formation. A few of the liver cells near to deposits of bacilli show mitotic figures. Leucocytes and lymphocytes are scanty. Many bacilli occur within blood-vessels, some free and some attached to desquamated endothelial cells.

Kidneys.

Bacilli occur in scanty numbers both in the interstitial tissue and within blood-vessels, but have not produced any lesions.

Spleen.

Bacilli are abundant, but have produced no definite tissue change.

SUMMARY.

The general condition is similar to that of a rabbit after intravenous infection with avian bacilli.

(4.)

VIRUS—A X.

Animal Inoculated.—Monkey 196.

Monkey 196 was inoculated intravenously with 10 mg. of culture of A X, and died 29 days afterwards.

Lungs.

Bacilli are distributed throughout the tissue in fairly large numbers. Compared with the lungs of Monkey 188, the differences noted in the present specimen are the following. Bacilli are not quite so numerous; there is less general thickening of the alveolar walls; there is more desquamation of epithelial cells.

Liver.

The general condition is the same as in the liver of Monkey 188. Bacilli are not quite so abundant. Mitoses in liver cells adjacent to deposits of bacilli are more frequent, the number of dividing nuclei being particularly large.

Kidneys.

Bacilli are present in small numbers in the interstitial tissue, inside glomeruli, and within blood-vessels. They have not produced any definite lesions.

Spleen.

Similar to the spleen of Monkey 188.

Mesenteric Gland.

Bacilli are very abundant, particularly within cells adjacent to the peripheral lymph sinus. The endothelial cells in which bacilli are contained are swollen, but there is no caseous change, and there is a noteworthy absence of tissue destruction, even in areas where bacilli are very abundant.

SUMMARY.

Similar to Monkey 188.

(5.)

VIRUS—A X.

Animal Inoculated.—Monkey 198.

Monkey 198 was inoculated intravenously with 100 mg. of culture of A X, and died 28 days afterwards.

Lungs.

Bacilli are distributed very abundantly throughout the tissue, including blood-vessels, and have grown into solid colonies and rosettes. There are no patches of consolidation or tissue destruction; the majority of the alveolar walls are not even thickened, but appear perfectly normal in structure, though they contain large numbers of bacilli. The absence of irritant action on the part of the bacilli is remarkable.

Liver.

The liver is crowded with dense masses of bacilli which have grown everywhere into colonies; the general appearance is identical with that of a rabbit's liver after intravenous inoculation. The liver cells are rather more broken down than in the liver of Monkey 188, but their nuclei have not disappeared and there is no caseation. Many of the liver cells show mitotic figures. In one case a few bacilli have been found within one of these cells; but this is exceptional, the rule being that cells containing bacilli do not undergo indirect nuclear division.

Kidneys.

Bacilli occur frequently in the interstitial tissue, the glomeruli, and the blood-vessels, and have often grown into small clumps. No definite lesions have been produced.

Spleen.

The spleen is almost a solid mass of bacilli. Rosette formations are abundant; a cell nucleus is generally found in the centre of the rosette. There is a remarkable absence of tissue destruction.

SUMMARY.

Similar to Monkey 188, but the bacilli have multiplied more abundantly.

General Results.

I. SUBCUTANEOUS INOCULATION.

As a rule the monkey has proved highly resistant to subcutaneous inoculation, and in consequence histological examination has given negative results. Monkey 118 is an exceptional case. This animal, killed 98 days after inoculation with 10 mg., shows acute disseminated tuberculosis which is identical in all histological respects with the disease produced by virulent mammalian bacilli.

II. INTRAVENOUS INOCULATIONS.

The animal inoculated with 1 mg. died in 34 days. In the tissues examined bacilli are scanty, and there are only a few lesions definitely attributable to their presence.

The animals inoculated with larger doses (10 to 100 mg.) died at earlier periods (18 to 29 days). In all these animals the bacilli have multiplied very abundantly and have often grown into typical rosettes, particularly in the liver and spleen. The amount of tissue damage is relatively small, and there is no formation of tubercles. The general features of the tissues resemble those produced in rabbits after intravenous inoculation with avian bacilli. It is also noticeable that, as with the rabbit and fowl, the liver and spleen of the monkey are more favourable sites than the lungs for the profuse multiplication of the avian bacillus.

V.—LESIONS IN SWINE.

(*a*) FEEDING EXPERIMENTS.

(1.)

VIRUS—P II.

Animal Fed.—Pig 110.

Pig 110 was fed with seven glycerin-serum tubes of culture of P II (a porcine virus with avian characters), and was killed 159 days afterwards.

Lungs.

There is some occasional thickening of the alveolar walls, but nothing has been found in the specimens examined which is suggestive of tuberculosis.

Mesenteric Gland.

There are large areas which are completely caseous and non-vascular; they are surrounded by a dense fibrous zone; no giant cells have been noted. Bacilli are scattered in the lesions, but are not numerous.

SUMMARY.

No evidence of dissemination.

(2.)

VIRUS—A VII.

Animal Fed.—Pig 176.

Pig 176 was fed with 50 mg. of culture of A VII, and was killed 315 days afterwards.

Lungs.

In the specimen of lung there are two retrogressive tubercles, but no bacilli have been found.

Liver, Kidneys, and Spleen.

No lesions or bacilli have been found in the specimens of these organs.

SUMMARY.

No bacilli have been found, but there are a few tubercles in the lungs.

(3.)

VIRUS—A VII.

Animal Fed.—Pig 178.

Pig 178 was fed once with 100 mg. of culture of A VII and was killed 101 days afterwards.

Lungs.

The tissue is congested, but in specimens not stained for bacilli there is nothing to suggest tuberculosis. Multinuclear leucocytes are not very numerous, but small lymphocytes are much in excess of the normal; very few plasma cells are present. In carbol-fuchsin specimens the tissue is seen to be crowded with bacilli which are uniformly distributed in all parts and have in some places assumed rosette formations characteristic of avian bacilli. Bacilli are numerous in the lymph spaces and occasionally occur within blood-vessels. The leucocytes very frequently contain bacilli.

Liver.

There is a general infiltration with lymphocytes and leucocytes. The leucocytes are highly phagocytic, many of them being crowded with bacilli. Numerous giant cells are present. There are no lesions with the definite structural arrangement of tubercles. Bacilli are present in all parts of the tissue, including the veins, in enormous numbers. Rosette formations are abundant and characteristic.

Kidneys.

There are no histological changes. In many of the glomeruli small groups of bacilli occur; a few are also found in the interstitial tissue.

Mesenteric Gland.

The gland is occupied by enormous masses of bacilli, which have produced very little tissue destruction. There is a very marked tendency for the bacilli to form rosettes. Numerous leucocytes contain bacilli.

SUMMARY

General dissemination and enormous multiplication of bacilli; very little tissue destruction; no tubercle formation. Many leucocytes contain bacilli.

(*b*) SUBCUTANEOUS INOCULATION.

VIRUS—A VII.

Animal Inoculated.—Pig 180.

Pig 180 was inoculated subcutaneously with 50 mg. of culture of A VII, and was killed 105 days afterwards.*

Lungs.

Throughout the tissue the alveolar walls show small irregular patches of thickening, but there are no definitely tubercular foci. Multinuclear leucocytes are in excess of the normal. Bacilli are distributed in small numbers throughout the tissue. They have not grown into colonies.

Liver.

There is a slight infiltration, chiefly interlobular, with leucocytes, lymphocytes, and plasma cells. A few giant cells are also present. Minute groups of bacilli occur in all parts of the tissue. There are no large groups and no rosette formations.

Kidneys.

There are no histological lesions, but bacilli are fairly numerous in some of the blood-vessels; none have been found elsewhere.

Spleen.

A few bacilli occur, but have not produced any definite tissue change.

Mesenteric Gland.

Bacilli are abundant and have assumed typical rosette formations. The gland is partially caseous. The leucocytes frequently contain bacilli.

SUMMARY.

Bacilli are present in all the tissues, but are not abundant except in the mesenteric gland, where they are very numerous and form typical rosettes. Relatively little tissue destruction has been caused.

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* Pig 264, subcutaneously inoculated with 50 mg. of A X and killed 105 days afterwards, shows neither lesions nor bacilli in the specimens examined of lungs, liver, kidneys, spleen, and a mesenteric gland.

(c) INTRAPERITONEAL INOCULATIONS.

(1.)

VIRUS—P II.

Animal Inoculated.—Pig 104.

Pig 104 received an intraperitoneal inoculation with 10 mg. of culture of P II (a porcine virus with avian characters), and was killed 69 days afterwards.

Liver.

The tissue contains several small lesions which are not caseous but contain giant cells and are infiltrated with numerous finely granular oxyphil leucocytes. Small lymphocytes are also present and a few plasma cells are found at the periphery of the lesions. The nuclei of the giant cells resemble those of the parenchymatous liver cells. Tubercle bacilli are present within the lesions but are rare.

Mesenteric Gland.

The gland is in an early stage of diffuse fibroid change. Giant cells are very numerous and appear to be formed from the endothelial cells covering the reticulum of the gland. There are no definitely caseous areas or circumscribed tubercles. There is a moderate degree of infiltration with oxyphil leucocytes. These cells often form small groups, particularly in situations where giant cell formation is going on. Bacilli are generally distributed, but occur in only small numbers. They are sometimes present within giant cells.

SUMMARY.

Bacilli are distributed in small numbers throughout the liver and mesenteric gland, and have produced lesions characterised by the presence of numerous giant cells.

(2.)

VIRUS—A VII.

Animal Inoculated.—Pig 182.

Pig 182 was inoculated intraperitoneally with 10 mg. of culture of A VII, and was killed 106 days afterwards.

Lungs.

There is a general and considerable thickening of the alveolar walls, but no definite tubercles have been formed. Bacilli are scattered all over the tissue and are found in nearly every microscopic field. They occur both singly and in small groups; no large colonies have been found. Leucocytes are distributed about the tissue but are not particularly numerous and do not seem to be attracted to the bacilli.

Liver.

The interlobular tissue shows patches of infiltration with lymphocytes and leucocytes; here and there these cells have invaded the parenchyma, and are then associated with partially breaking down liver cells. and imperfectly formed giant cells. There are no definitely formed tubercles. Bacilli are abundant and are scattered all over the tissue. They are found in all the areas of infiltration and in the giant cells, and also occur in all parts of the tissue where these is no sign of histological change. Groups of bacilli rolled into solid balls about the size of a red corpuscle are frequently found in the capillary channels or attached to their walls.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Bacilli are distributed throughout the tissue and in many places are numerous, but have not formed solid colonies. In some places the cells where the bacilli occur stain faintly but are not definitely caseous; in the greater part of the tissue the bacilli are not associated with any histological change. Leucocytes are not particularly numerous and do not seem attracted towards the bacilli.

Mesenteric Gland.

The greater part of the gland has been replaced by bacilli, but islands of lymphoid tissue are left. The colonies show the typical rosette formation. The leucocytes very frequently contain bacilli.

SUMMARY.

The bacilli are generally disseminated, except in the kidneys, and have multiplied freely. They have caused very little tissue destruction.

(d) INTRAVENOUS INOCULATIONS.

(1.)

VIRUS—A X.

Animal Inoculated.—Pig 236.

Pig 236 was inoculated intravenously with 5 cc. of an emulsion of the mesenteric gland of Calf 566 (inoculated with A X), and died 120 days afterwards.

Lungs.

The tissue is crowded with bacilli, which have grown everywhere into colonies and rosettes. There are no areas which are otherwise histologically typical of tuberculosis; but in patches where the collections of bacilli are very large, the alveolar walls are greatly thickened and show an increase of endothelial cells or fibroblasts; there are some lymphocytes in these areas, but leucocytes are very scanty. The bacilli are not confined to these thickened patches, but are growing freely amongst otherwise normal alveolar walls, the total area of which is largely in excess of the consolidated tissue.

Liver.

The tissue is crowded with bacilli, which have grown everywhere into colonies and rosettes. The bacilli have produced relatively little tissue destruction, and there is an absence of caseation and tubercle formation. The bacilli show a marked tendency to intracellular growth, and have multiplied with particular abundance within parenchymatous cells, giving rise to small multinucleate masses or giant cells.

Kidneys.

Compared with the liver, bacilli are very scanty, but minute groups and colonies are found here and there within glomeruli and in the intertubular tissue. They have produced no appreciable tissue change.

Spleen.

Though much less abundant than in the liver, bacilli are distributed throughout the tissue in large numbers, and have produced minute patches of slight cellular disintegration.

A Bronchial and a Mesenteric Gland.

These glands are occupied by an almost continuous growth of bacilli. The individual colonies and rosettes are particularly large. There is very little tissue destruction. Giant cells are numerous. Leucocytes are scanty.

SUMMARY.

Enormous multiplication of bacilli throughout the body. Very little tissue destruction and no tubercle formation.

(2.)

VIRUS—A VIII.

Animal Inoculated—Pig 248.

Pig 248 was inoculated intravenously with 50 mg. of culture of A VIII, and died 47 days afterwards.

Specimens of lung, liver, kidneys, spleen, and a lymphatic gland (portal) are almost identical with the corresponding organs of Pig 236. In the liver there is rather more tissue destruction, with small patches of commencing caseation, and leucocytes are rather more numerous. Similar differences are observable in the spleen. It has also been noted that in the liver and spleen leucocytes contain bacilli more frequently than in the corresponding tissues of Pig 236. In the lymphatic gland, also, the bacilli have produced more tissue disintegration and leucocytes are very numerous.

SUMMARY.

Similar to Pig 236 but rather more tissue destruction, except in the lungs, and more leucocytes.

General Results.

I. FEEDING.

Pigs 110 and 176 were both fed with large doses, but I have found no evidence of dissemination in the former animal and in the latter only a few, obviously retrogressive, tubercles of the lungs, in which no bacilli are demonstrable.

Pig 178, though fed with the same virus as Pig 176, but with 100 mg. instead of 50 mg., shows a remarkable difference, the general condition indicating a very free circulation of bacilli by the blood-stream. Though the bacilli are generally disseminated and have multiplied very abundantly, they have caused very little tissue destruction, and have not led to the formation of tubercles.

II. SUBCUTANEOUS INOCULATIONS.

Pig 180, killed 105 days after subcutaneous inoculation with 50 mg., shows a free distribution of bacilli in the various tissues examined, and there are indications that the organisms are circulating in considerable numbers in the blood-stream. Relatively little tissue destruction has been caused. The bacilli do not show signs of copious multiplication except in a mesenteric gland, where they are very numerous and form typical rosettes.

Pig 264, inoculated with the same dose and killed after the same period, shows neither lesions nor bacilli.

III. INTRAPERITONEAL INOCULATIONS.

Pig 104 received a dose of 10 mg., and shows a distribution of bacilli in small numbers throughout the liver and a mesenteric gland. The bacilli have not led to the formation of organised tubercles, but have caused the production of large numbers of giant cells.

Pig 182, inoculated with the same dose as the former animal (10 mg.), shows a general dissemination and free multiplication of bacilli in the lungs, liver, spleen, and a mesenteric gland, but not in the kidneys. There is very little tissue destruction and no tubercle formation, but in the liver there are several imperfectly formed giant cells.

IV. INTRAVENOUS INOCULATIONS.

Pigs 236 and 248 show general dissemination and enormous multiplication of bacilli, with relatively little tissue destruction.

VI.—LESIONS IN GUINEA-PIGS.

(a) SUBCUTANEOUS INOCULATIONS.

(1.)

VIRUS—P II.

Animal Inoculated.—G.P. 1532

Guinea-pig 1532 was inoculated subcutaneously with 1 mg. of culture of P II (a porcine virus with avian characters), and died 54 days afterwards.

Lungs.

No tubercle bacilli have been found, and no lesions suggestive of tuberculosis.

Liver.

There are numerous foci which are partially caseous and are overrun with leucocytes and lymphocytes. No tubercle bacilli have been found.

Kidneys and Spleen.

No lesions or bacilli have been found.

SUMMARY.

Neither tubercle bacilli nor typical tubercles have been found.

(2.)

VIRUS—A V.

Animal Inoculated.—G.P. 1962.

Guinea-pig 1962 was inoculated subcutaneously with tissue emulsion of liver of A V (a pheasant), and was killed 109 days afterwards.

Lungs.

The alveolar walls show patches of congestion and cellular infiltration, and there are several areas of consolidation. Some of these areas are large (twice the diameter of the microscopic field ; low-power), slightly caseous, and appear typical of tuberculosis. They contain tubercle bacilli in small numbers. In the smaller areas, which are filled up with lymphocytes, no tubercle bacilli have been found.

Liver.

A few small foci are present which are centrally caseous and are surrounded by lymphocytes and fibroblasts. No bacilli have been found in them.

Kidneys and Spleen.

No lesions or bacilli have been found.

Portal Gland.

Caseous areas are present and contain bacilli in rather scanty numbers.

SUMMARY.

Disseminated lesions of a chronic type are present. Bacilli are scanty.

(b) INTRAPERITONEAL INOCULATIONS.

(1.)

VIRUS—P II.

Animal Inoculated.—G.P. 1494.

Guinea-pig 1494 received an intraperitoneal inoculation with a whole culture of P II (a porcine virus with avian characters), and was killed 37 days afterwards.

Lungs.

There are several small patches of consolidated alveoli. The cells in some of these areas appear to be slightly necrotic, but there is no definite caseation. Some of the areas are infiltrated with lymphocytes and leucocytes. No giant cells are present. The areas referred to have no definite boundary zone. Tubercle bacilli are present in small numbers in these areas; many of them are fragmentary, and some show globular swellings.

Liver.

Both within and between the lobules there are numerous small patches of infiltration with lymphocytes and leucocytes. In most of these situations a few tubercle bacilli are present.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Bacilli are present in scanty numbers and have produced slight necrotic change.

Mesenteric Gland.

Many small groups of bacilli are present. They have produced very little tissue change.

SUMMARY.

The bacilli are generally disseminated ; they appear to be dying out, and have produced only slight tissue change.

(2.)

VIRUS—P II.

Animal Inoculated.—G.P. 1533.

Guinea-pig 1533 was inoculated intraperitoneally with 5 mg. of culture of P II (a porcine virus with avian characters), and died 19 days afterwards.

Lungs.

There is a general congestion of the alveolar walls, which are infiltrated with leucocytes and lymphocytes, and much of the alveolar epithelium has desquamated. Throughout the tissue there are small groups of tubercle bacilli; they are generally attached to, or within, epithelial and endothelial cells and have often grown into small groups within both these types of cells. A few bacilli, adherent to endothelial cells, are present within blood vessels. There is no formation of definite tubercles.

Liver.

The liver is in a condition of diffuse necrotic change. Throughout the tissue tubercle bacilli are distributed, but nowhere form large groups. A few giant cells are present but there are no definite tubercles.

Kidneys.

Some interstitial infiltration is present, but there are no definite tubercles. A few bacilli have been found, both amongst the interstitial tissue and within glomeruli.

SUMMARY.

Dissemination of the bacilli in the lungs, without the production of tubercles; advanced necrotic change in the liver.

(3.)

VIRUS—A IV.

Animal Inoculated.—G.P. 2131.

Guinea-pig 2131 received an intraperitoneal inoculation with 1 mg. of culture of A IV, and was killed 180 days afterwards.

Lungs, Kidneys, Spleen, and Mesenteric Gland.

No tubercles or bacilli have been found.

Liver.

There are several small foci containing numerous multinuclear leucocytes, and sometimes showing evidence of slight caseation. No bacilli have been found.

SUMMARY.

Neither tubercle bacilli nor definite tubercles have been found. The condition of the liver suggests that tubercle bacilli may have reached that organ, but have been destroyed.

(4.)

VIRUS—A VI.

Animal Inoculated.—G.P. 2624.

Guinea-pig 2624 was inoculated intraperitoneally with ·1 mg. of culture of A VI, and died 57 days afterwards.

Lungs.

The lungs contain small lesions. Each is composed of a small group of swollen and partially broken-down epithelial and endothelial cells, together with an infiltration of leucocytes and lymphocytes. Bacilli are rare.

Liver.

There is a general infiltration of lymphocytes and leucocytes, and there are many places where these cells are collected in a group amongst a patch of slightly disintegrated epithelial and endothelial cells. In some of these foci a few bacilli are present. Some of the liver cells show mitotic figures.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The spleen contains small, slightly caseous foci and some giant cells. Bacilli are scanty.

SUMMARY.

Small, disseminated lesions. Bacilli scanty.

(5.)

VIRUS—A VIII.

Animal Inoculated.—G.P. 2503.

Guinea-pig 2503 was inoculated intraperitoneally with 1 mg. of culture of A VIII, and died 52 days afterwards.

Lungs.

Small groups of bacilli are found in many parts of the tissue. They have not given rise to definite tubercles but have caused a swelling up of the endothelial cells and small aggregations of leucocytes and lymphocytes.

Liver.

Bacilli occur in small numbers all over the tissue. There are no definite tubercles or areas of caseation, but the parenchymatous cells are extensively damaged; their protoplasm stains badly and their nuclei are distorted. The tissue as a whole is overrun with leucocytes and lymphocytes, and there is a large number of cells with oval, pale, and swollen nuclei which are apparently representatives of endothelial and parenchymatous cells. A few giant cells are present. The general characteristics of the cells found in the tissue are confusion and partial disintegration.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are several small, caseous patches, in which bacilli are present in scanty numbers.

SUMMARY.

Bacilli are distributed in small numbers in the lungs and liver. No definite tubercles have been formed, but in the lungs there are small patches showing commencing degeneration of the tissue cells, and the liver parenchyma is extensively damaged.

General Results.

The preceding examples show that the tissues of the guinea-pig are highly resistant to the avian tubercle bacillus, both after subcutaneous and after intraperitoneal inoculation. There is frequently evidence of dissemination; but the lesions, though sometimes caseous, are on the whole not typical of tuberculosis, and the bacilli generally seem unable to multiply or to retain their vitality in the organs of this animal. But in some cases inoculation has been followed by death at a comparatively early period; and the evidence of irritant and necrotic change in the liver suggests that the bacilli may have exerted a toxic effect.

VII.—LESIONS IN GOATS.

(*a*) SUBCUTANEOUS INOCULATIONS.

(1.)

VIRUS—P II.

Animal Inoculated.—Goat 36.

Goat 36 was inoculated subcutaneously with 10 mg. of culture of P II (a porcine virus with avian characters), and was killed 131 days subsequently.

Lungs.

The lungs contain circumscribed, caseous lesions in which giant cells are present. Tubercle bacilli are very rare, but a few have been found. In another portion of the lung numerous parasitic worms have been found, but these are not associated with either caseous lesions, giant cells, or tubercle bacilli.

Mesenteric Gland.

The gland examined contains extensive fibro-caseous lesions, with many giant cells. Tubercle bacilli are scanty.

SUMMARY.

Some bacilli have reached the lungs, and have there set up chronic, probably retrogressive, tubercles.

(2.)

VIRUS—A III.

Animal Inoculated.—Goat 40.

Goat 40 was inoculated subcutaneously with 100 mg. of culture of A III, and was killed 128 days afterwards.

Lungs.

Necrotic lesions are present and are surrounded by a dense leucocytic infiltration. One or two tubercle bacilli are present. No other parasites have been found, but it does not seem quite certain that the lesions have been produced by the tubercle bacilli.

Other Organs.

No tubercles or bacilli have been found in the specimens of liver, kidneys, or spleen. In the prescapular gland there are a few suspicious foci, but no bacilli have been found in them.

SUMMARY.

Lesions of doubtful nature, but containing a few tubercle bacilli, are present in the lungs. There is no clear evidence of dissemination elsewhere.

(3.)

VIRUS—A X.

Animal Inoculated.—Goat 42.

Goat 42 was inoculated subcutaneously with 50 mg. of culture of A X, and was killed 98 days afterwards.

Lungs.

In the specimen of lung there are some small patches of consolidation. No bacilli have been found in them, and there is no definite clue to their origin.

Other Organs.

No evidence of infection has been found in specimens of liver, kidney, spleen, and a popliteal gland.

SUMMARY.

No evidence of dissemination.

(4.)

VIRUS—A X.

Animal Inoculated.—Goat 48.

Goat 48 was inoculated subcutaneously with 50 mg. of culture of A X, and was killed, when ill, 83 days afterwards.

Lungs.

The tissue is occupied with numerous caseous, semi-confluent tubercles; the larger tubercles contain a little calcareous deposit in the centre. The alveoli between these foci contain smaller lesions which have not commenced to caseate. Parasitic worms (strongyli) are present in some of the bronchioles and alveoli, but do not appear to have any causal relationship to the tubercles. Bacilli are present in all the lesions and are very abundant in the larger lesions, both in the interior and at the periphery; at the periphery they have grown into colonies and rosettes.

Liver.

Bacilli are scattered throughout the tissue in large numbers and have grown into colonies and rosettes. They have not formed tubercles but show a marked tendency to grow within the parenchymatous cells. Cells containing colonies of bacilli occur in groups; they take a diffuse protoplasmic and nuclear stain, and consequently stand out conspicuously from the adjacent tissue.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Bacilli are abundant and have grown into rosettes. There are many patches of commencing caseation, containing many broken down leucocytes.

Mesenteric Gland.

The section may be described as a growth of bacilli, amongst which several large islands of lymphoid tissue have survived. Rosette formations are very numerous. Only fragments of nuclear material have survived amongst the denser growth of bacilli.

Intestine.

A section of small intestine is swarming with bacilli which have grown into rosettes. Many of the villi are broken down, but there are no caseous deposits, and the amount of tissue destruction is small relatively to the enormous numbers of bacilli.

SUMMARY.

General dissemination, except in the kidneys, and great multiplication of bacilli. In the lungs there are large, progressive, caseous lesions; elsewhere, the amount of tissue destruction is relatively small.

(*b*) INTRAVENOUS INOCULATION.

(1.)

VIRUS—P III.

Animal Inoculated.—Goat 56.

Goat 56 was inoculated intravenously with 10 mg. of culture of P III (a porcine virus with avian characters), and died 40 days afterwards.

Lungs.

There is a partial consolidation due to a generalised thickening of the alveolar walls, but there are no lesions typical of tuberculosis. Bacilli are abundant in all parts of the tissue, and form small clumps; they have evidently multiplied.

Liver.

Bacilli are numerous; they are scattered throughout the tissue, and have often grown into small colonies. They have produced very little tissue destruction, and have not led to the formation of definite tubercles; there is some infiltration of leucocytes and lymphocytes in their neighbourhood.

Kidneys.

No lesions or bacilli have been found.

Spleen.

A few bacilli are present, but no lesions have been produced.

Mediastinal Gland.

There are large patches of bacilli, which have grown into colonies and rosettes. Very little tissue damage has been produced.

SUMMARY.

General dissemination of bacilli, with abundant multiplication in the lungs, liver, and a mediastinal gland. Very little tissue damage.

(2.)

VIRUS—P III.

Animal Inoculated.—Goat 58.

Goat 58 was inoculated intravenously with 1 mg. of culture of P III (a porcine virus with avian characters), and died 35 days afterwards.

Lungs.

The histological condition is identical with that of the lungs of Goat 56. Bacilli, though plentiful, are not so numerous as in the latter animal.

Liver.

Identical with the liver of Goat 56.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Bacilli are very rare; no lesions have been produced.

Mediastinal Gland.

Similar to the corresponding gland of Goat 56, but bacilli less abundant.

SUMMARY.

The bacilli have not multiplied quite so abundantly as in Goat 56, but otherwise the conditions of the two animals are identical.

General Results.

I. SUBCUTANEOUS INOCULATIONS.

The results are irregular. The first three animals proved highly resistant. In Goats 36 and 40, the only evidence of dissemination is in the lungs, which, in each case, contain a few bacilli; these are associated in the former animal with caseous lesions and giant cell formation; in the other animal the lesions are of doubtful nature. No evidence of dissemination has been found in Goat 42. But in Goat 48, inoculated with the same dose of the same virus, the bacilli are generally disseminated and have multiplied abundantly. The lungs contain large, progressive, caseous lesions, but in the other organs the amount of tissue destruction is small and there are no tubercles. The bacilli have produced typical rosette formations.

II. INTRAVENOUS INOCULATIONS.

In the two animals examined there is a general dissemination and abundant multiplication of bacilli, but no tubercle formation and very little tissue damage.

VIII.—LESIONS IN RATS.

(*a*) SUBCUTANEOUS INOCULATIONS.

(1.)

VIRUS—A II.

Animal Inoculated.—Rat 122.

Rat 122 was inoculated subcutaneously with 100 mg. of culture of A II, and was killed 255 days afterwards.

Lungs.

No bacilli or lesions suggestive of infection have been found.

Liver.

There are a few minute lesions of doubtful nature. Two or three tubercle bacilli have been found amongst the interlobular tissue.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are severa minute foci, each consisting of a group of about half a dozen partially disintegrated cells. Some of these foci contain a tubercle bacillus.

SUMMARY.

Slight evidence of dissemination. Bacilli very rare.

(2.)

VIRUS—A IV.

Animal Inoculated.—Rat 86.

Rat 86 was inoculated subcutaneously with 4 mg. of culture of A IV, and died 333 days afterwards.

Lungs.

There are several small foci which are not caseous but show aggregations of lymphocytes and some leucocytes. In these areas bacilli are present in fairly large numbers.

Liver and Spleen.

There are no definite lesions, but a few bacilli have been found.

Kidneys.

Neither lesions nor bacilli have been found.

SUMMARY.

Very little tissue change. Bacilli fairly numerous in the lungs, scanty elsewhere.

(*b*) INTRAPERITONEAL INOCULATIONS.

(1.)

VIRUS—P II.

Animal Inoculated.—Rat 85.

Rat 85 received an intraperitoneal inoculation with 10 mg. of culture of P II (a porcine virus with avian characters), and was killed 184 days afterwards.

Lungs.

The tissue shows no very definite lesions, but here and there the alveolar walls have been invaded with small groups of cells which are mainly lymphocytes; and in Pappenheim specimens many small groups of plasma cells are demonstrable. No fibrin is present. Throughout the tissue small groups of bacilli are present. They are frequently intracellular and are associated with the aggregations of lymphocytes noted above, though they also occur (often within epithelial and endothelial cells) where no cellular infiltration is present. A few have been found within blood-vessels and in the endothelial lining of blood-vessels. The bacilli vary from about 1 to 3·5 μ in length. Many are fragmentary and some are beaded. Several of the longer forms are curved.

Liver.

There is very little tissue change, with the exception of a moderate amount of leucocytic infiltration. Bacilli are very abundant, especially in the interlobular tissue, where they are universally distributed and form clusters of small colonies. Bacilli also occur within the lobules, though less plentifully. The bacilli have a marked tendency to intracellular growth; cells are frequently found the protoplasm of which is completely filled with them. The bacilli are mostly straight and from ·75 to 2·5 μ in length: many of the longer forms are beaded or irregularly stained.

Kidneys.

In a small area of the section examined there is some leucocytic infiltration and many of the renal tubules are filled with leucocytes; the rest of the section appears histologically normal. In the infiltrated area referred to there are dense masses of bacilli, which, entangled amongst leucocytes, occupy the interior of many of the tubules; and, in the adjacent area, bacilli are found, though less plentifully, in the interstitial tissue. No bacilli have been found in other parts of the section.

Axillary Gland.

Tubercle bacilli are fairly abundant, but have produced very little tissue change. The gland is also occupied with numerous colonies of cocci. These occur in different situations from the bacilli. The two organisms are not found growing in association with each other.

SUMMARY.

The tissue is swarming with bacilli, which have a marked tendency to intracellular growth. There is some leucocytosis but very little tissue destruction and no tubercle formation.

(2.)

VIRUS—P III.

Animal Inoculated.—Rat 79.

Rat 79 received an intraperitoneal inoculation with 1 mg. of culture of P III (a porcine virus with avian characters), and died 219 days afterwards.

Lungs.

A few small groups of lymphocytes and plasma cells are present in the alveolar walls, and there is a well marked layer of plasma cells at the periphery of the bronchioles. No other lesions are present. In the small alveolar foci a few tubercle bacilli are present. They are minute, often fragmentary, and almost always intracellular.

Liver.

No tubercles or other definite lesions are present, but plasma cells are numerous in the interlobular tissue and are also distributed freely, though not in groups, within the lobules. Lymphocytes and leucocytes are also more numerous than normal. Tubercle bacilli form minute colonies in all parts of the tissue. The bacilli are nearly all intracellular. Lymphocytes, leucocytes, and plasma cells are always to be found somewhere near the bacilli but have not formed definite aggregations in the situations where bacilli are present.

Kidneys.

No lesions or bacilli have been found.

Mediastinal Gland.

More than half the gland is completely necrotic, and in the cellular portion there are found numerous areas which stain rather faintly. These pale areas are occupied with colonies of bacilli. In the completely necrotic areas bacilli are scanty, but colonies of them are to be found wherever any groups of cell fragments remain.

Spleen.

Bacilli are distributed throughout the spleen in small groups. They have caused no tissue destruction.

SUMMARY.

It is noteworthy that these bacilli, given in a relatively small dose for a rat, have not only survived for 219 days but have multiplied abundantly in the liver and mediastinal gland.

(3.)

VIRUS—A I.

Animal Inoculated.—Rat 102.

Rat 102 received an intraperitoneal inoculation with 1 mg. of culture of A I, and was killed 109 days afterwards.

Lungs.

A few bacilli are present and are associated with small aggregations of cells which are mainly lymphocytes.

Liver.

Several bacilli, sometimes in small groups, are present. There is a tendency for them to be surrounded by fibroblasts, but the lesions cannot be regarded as definite tubercles.

Kidneys.

No lesions or bacilli have been found.

Spleen

Bacilli are distributed in moderately large numbers throughout the tissue. In many situations they have produced pale areas in which the only conspicuous elements are cells resembling young fibroblasts or endothelial cells. There is no indication that the bacilli are multiplying.

SUMMARY.

There is evidence of dissemination but, except in the spleen, bacilli are not numerous. They have caused only slight tissue change.

(4.)

VIRUS—A VII.

Animal Inoculated.—Rat 125.

Rat 125 was inoculated intraperitoneally with 1 mg. of culture of A VII, and was killed 177 days afterwards.

Lungs.

There is only slight evidence of infection. A few bacilli are present and are occasionally associated with partial cell fusions, but there are no definite tubercles or giant cells.

Liver.

There are many small foci consisting of partially disintegrated liver cells and a small collection of lymphocytes and leucocytes. In many of these lesions bacilli are fairly numerous; they also occur singly in many parts of the tissue showing no histological change.

Kidneys.

No lesions or bacilli have been found.

Spleen.

The spleen contains several small, slightly caseous foci in which bacilli are scanty.

SUMMARY.

The bacilli have disseminated, but have not multiplied freely, and have produced only slight tissue change. The liver appears to be the most susceptible organ.

(5.)

VIRUS—A VII.

Animal Inoculated.—Rat 127.

Rat 127 was inoculated intraperitoneally with 20 mg. of culture of A VII, and was killed 82 days afterwards.

Lungs.

There are no definite tubercles, but bacilli are moderately numerous and occur in all parts of the specimen. There is a general infiltration of wandering cells, amongst which lymphocytes are much more numerous than leucocytes. Round some of the deposits of bacilli these cells are collected in large numbers.

Liver.

The condition of the liver is identical with that of Rat 125, with the exception that bacilli are rather more numerous.

Kidneys.

Between the tubules there is a slight degree of infiltration of lymphocytes and leucocytes. Bacilli are generally distributed but are less numerous than in the liver. Some of the bacilli are found within glomeruli.

Spleen.

Bacilli are generally distributed and have grown into small groups. There is a slight tendency to caseation in some of the places where groups of bacilli occur.

Bronchial Gland.

Masses of bacilli are present in many parts of the gland. Where they occur the cells stain faintly, but there is only slight caseation. Many of the bacilli are broken up. There is no rosette formation.

SUMMARY.

Generally dissemination, similar to that found in Rat 125, but with more abundant multiplication of bacilli.

(6.)

VIRUS—A VIII.

Animal Inoculated.—Rat 133.

Rat 133 was inoculated intraperitoneally with 1 mg. of culture of A VIII, and was killed 497 days afterwards.

Lungs, Liver, and Kidneys.

Neither lesions nor bacilli have been found in the specimens examined.

Spleen.

There are a few small lesions, not definitely tubercular, in which bacilli occur in scanty numbers.

SUMMARY.

Slight evidence of dissemination in the spleen.

General Results.

I.—SUBCUTANEOUS INOCULATIONS.

Although Rat 122 received a very large dose (100 mg.), the animal proved highly resistant. There are a few bacilli in the liver and spleen, but there is very little tissue damage. In Rat 86 bacilli are fairly numerous in the lungs, though the dose was much smaller.

II.—INTRAPERITONEAL INOCULATIONS.

Most of the animals, even after the relatively small dose of 1 mg., show general dissemination of bacilli but an absence of tubercle formation. In all except two of the animals the bacilli have been able to multiply abundantly in some of the organs The bacilli have multiplied less freely in the lungs than in the liver, spleen, and lymphatic glands.

IX.—LESIONS IN OTHER ANIMALS.

(1.)

VIRUS—P II.

Animal Inoculated.—Cat 62.

Cat 62 received an intraperitoneal inoculation of 1 mg. of culture of P II (a porcine virus with avian characters), and died 73 days afterwards.

Lungs.

The alveolar walls show many small patches of thickening, in which the epithelial cells are swollen and a few leucocytes and lymphocytes are present. There is no caseation and no histological evidence of tuberculosis. No fibrin is present. Bacilli are richly distributed throughout the tissue, forming small colonies and groups of colonies visible everywhere with a low power. Some of the colonies have grown in the form of a rosette. The bacilli are very frequently intracellular, and have a tendency to wrap themselves round cell nuclei. Bacilli are also frequently found within blood-vessels. The bacilli are uniformly stained, straight, and on the whole very minute, many of them measuring not more than ·5 μ in length. Forms as long as 2·5 or 3 μ occur, but are rare, and the average length is not more than 1 μ. There is very little tissue reaction relatively to the large number of bacilli present.

Liver.

Numerous colonies of bacilli are scattered throughout the liver substance. The bacilli have produced no necrosis and no proliferation of endothelial cells, connective tissue cells, or parenchymatous cells, but there is an abundant infiltration of small lymphocytes and leucocytes (chiefly the former), and some proliferation of bile ducts. The absence of any zone of connective tissue or other cells at the periphery of the foci occupied by colonies of bacilli is noteworthy. In the centre of many of the foci the walls of the blood capillaries have broken down, and there is an irregular group of red blood corpuscles without any definite boundary. A large proportion of the bacilli are intracellular. Both cells of the liver parenchyma and endothelial cells are often filled with them. Bacilli are also found within large lymphocytes and leucocytes. In addition to the definite foci, marked by histological change, where the bacilli occur in colonies, bacilli are found in small numbers in the rest of the tissue. They very frequently occur within cells of the liver parenchyma, and are sometimes found within blood-vessels. Morphologically the bacilli resemble those found in the lungs.

Kidneys.

No histological lesions have been found. A few bacilli occur in some of the blood-vessels, but none have been found in other situations.

Spleen.

The spleen contains dense masses of bacilli, which show a marked tendency to intracellular growth. Many bacilli are present within blood-vessels.

Sternal Gland.

In specially stained specimens the gland appears to be almost a solid mass of bacilli. Their tendency to intracellular growth is very conspicuous. The protoplasm of the majority of the cells is entirely replaced by bacilli; but the nuclei have not been attacked and still retain their staining properties. In specimens not stained for bacilli the absence of tissue destruction, or anything in the nature of tubercle formation, is very notable.

Omentum.

A patch of greatly thickened omentum has been examined and is found to consist of an almost solid mass of bacilli. The relation of bacilli to cells is the same as in the sternal gland.

SUMMARY.

The tissues, except the kidneys, are swarming with bacilli, which have a marked predilection for intracellular growth, and have caused very little tissue destruction.

(2.)

VIRUS—P III.

Animal Inoculated.—Dog 122.

Dog 122 (pup) was inoculated subcutaneously with 25 mg. of culture of P III (a porcine virus with avian characters), and was killed 74 days afterwards.

Lungs.

In one part of the specimen there is a caseous nodule which is surrounded by connective tissue corpuscles and plasma cells. Within the nodule there are a few tubercle bacilli. Neither lesions nor bacilli have been found elsewhere.

Liver.

In the specimen examined there is one focus where the liver cells are swollen and distorted and are infiltrated with a small number of lymphocytes. In this area one or two tubercle bacilli have been found, and there are a few fragments of fibrin.

Kidneys and Spleen.

No lesions or bacilli have been found.

Iliac Gland.

There are no histological lesions, but bacilli are present in small numbers and are almost all intracellular.

SUMMARY.

There has been some dissemination in the lungs and liver, but very few bacilli have survived and only a small amount of tissue change has been produced.

(3.)

VIRUS—A X.

Animal Inoculated.—Dog 162.

Dog 162 (a pup) was inoculated intravenously with 50 mg. of culture of A X, and was killed 146 days afterwards.

Lungs.

There is some general thickening of the alveolar walls and there are several small, suspicious foci, none of which are definite enough to be called tubercles. No bacilli have been found.

Liver.

Throughout the tissue there are numerous minute foci where a group of liver cells is partially damaged and infiltrated with adventitious cells. Three tubercle bacilli have been found in these situations.

Kidneys.

No tubercles or bacilli have been found.

SUMMARY.

The bacilli have almost entirely disappeared, but have left behind minute, retrogressive lesions.

(4.)

VIRUS—P III.

Animal Inoculated.—Mouse 67.

Mouse 67 received an intraperitoneal inoculation with 1 mg. of culture of P III (a porcine virus with avian characters), and was killed 41 days afterwards.

Lungs.

A few very small groups of bacilli are present here and there. Some of them are associated with small collections of lymphocytes and leucocytes, but in many instances the bacilli have produced no tissue change.

Liver.

In the tissue between the lobules, bacilli are fairly numerous and have grown into small groups. In these situations there is an infiltration of cells which are chiefly lymphocytes. The bacilli have also made their way in small numbers into the interior of the lobules and are often found either within or adherent to liver cells.

Kidneys.

No lesions or bacilli have been found.

Spleen.

Bacilli are scattered throughout the organ, and in many places have grown into small groups. No evidence of tissue destruction has been produced.

SUMMARY.

Bacilli are multiplying in the liver and spleen, though with the production of only slight tissue change. They appear to find the lungs a less favourable situation for their growth.

(5.)

VIRUSES—A IV and A X.

Animals Inoculated.—Mice 84, 88, and 105.

These three animals were inoculated subcutaneously. The first received 1 mg. of A IV and died 200 days afterwards, the second 20 mg. of A IV and died 143 days afterwards, the third 50 mg. of A X and died 105 days afterwards.

Specimens of the lungs, livers, kidneys, spleens, and lymphatic glands have been examined. All three animals present very much the same appearances. The organs are swarming with bacilli, but very little tissue destruction has been produced The lungs, in particular, present a solid mass of bacilli, and it is difficult to understand how the animals were able to breathe. In all three cases the free multiplication of bacilli must have been attended by relatively little pathogenic effect. The amount of dose seems to have had some effect on the duration of life ; and with regard to the livers, where the bacilli are not so densely packed as in the lungs, it is noticeable that the growth of bacilli is most copious in the animal which received the smallest dose and lived the longest time.*

SUMMARY.

Dissemination and enormous multiplication of bacilli in all the organs of the body, with very little tissue change.

(6.)

VIRUS—P III.

Animal Fed.—Pony 14.

Pony 14 was fed with 600 mg. of culture of P III (a porcine virus with avian characters), and was killed 138 days afterwards.

Lungs.

No bacilli have been found, and no lesions suggestive of tuberculosis.

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* Mouse 94, which died 27 days after intraperitoneal inoculation with 10 mg. of A IV, is in a similar condition to the above animals. Mouse 87, which died 277 days after subcutaneous inoculation of 10 mg. of A IV, shows fewer bacilli in the liver and none in the kidneys. Mouse 90, which died 280 days after subcutaneous inoculation with 40 mg. of A IV, resembles Mouse 87.

Liver.

There are some small lesions which are undergoing fibroid change and are infiltrated with lymphocytes. These might have been due to tubercle bacilli, but none have been found.

Kidneys and Spleen.

No lesions or bacilli have been found.

Mesenteric Gland.

There are many small caseous lesions which are of a chronic type and contain numerous giant cells. Bacilli are present but rare.

SUMMARY.

No definite evidence of dissemination beyond the nearest glands.

(7.)

VIRUS—A VII.

Animal Inoculated.—Pony 10.

Pony 10 was inoculated subcutaneously with 100 mg. of culture of A VII, and died 38 days afterwards.

Lungs.

There are no definite lesions, and no bacilli have been found.

Liver.

No tubercle bacilli have been found. Patches of leucocytic infiltration are present, but these are associated with blue-staining bacteria.

Kidneys.

No lesions or bacilli have been found.

Spleen.

There are several small deposits of bacilli which have produced slight caseous change.

Prescapular Gland.

Bacilli are very abundant. They often occur within giant cells, forming a ring surrounded by a peripheral row of nuclei. There are no definite tubercles. The main feature of the section is the enormous number of multinucleate cell fusions and giant cells.

SUMMARY.

With the exception of the spleen there is no evidence of dissemination beyond the nearest gland.

(8.)

VIRUS—A VIII.

Animal Inoculated.—Pony 2.

Pony 2 was inoculated subcutaneously with 50 mg. of culture of A VIII, and was killed 158 days afterwards.

Neither tubercle bacilli nor histological evidence of dissemination has been found in the specimens of lungs, liver, kidneys, and spleen.

SUMMARY.

No evidence of dissemination.

(9.)

VIRUS—A X.

Animal Inoculated.—Pony 32.

Pony 32 was inoculated intravenously with 10 mg. of culture of A X, and died 15 days afterwards.

Lungs.

The tissue is congested and there is much desquamation of epithelial cells, but there are no localised lesions. Tubercle bacilli are present in scanty numbers. Other bacilli are much more numerous.

Liver.

Tubercle bacilli are present in scanty numbers, but have produced no definite lesions.

Kidneys.

There are no definite lesions but a few tubercle bacilli occur, both in the glomeruli and in the interstitial tissue.

Spleen.

A few tubercle bacilli are present, but have not produced any lesions.

Prescapular Gland.

A few minute caseous foci and giant cells are present, but no bacilli have been found.

SUMMARY.

The bacilli are disseminated in small numbers but show no signs of multiplication, or of producing progressive disease.

General Results.

Cat 62.—In this animal, after the small intraperitoneal dose of 1 mg., the tissues are swarming with bacilli, which have a marked predilection for intracellular growth and have caused very little tissue destruction.

Dog 122 shows a high resistance to 25 mg. inoculated subcutaneously. The bacilli have produced a few slight, retrogressive lesions in the lungs and liver.

Dog 162 was inoculated intravenously with 50 mg. In most species of animals the avian bacillus multiplies abundantly after intravenous inoculation, but in this case the bacilli have almost entirely disappeared, and have left behind only minute, retrogressive lesions.

Mouse 67 has reacted in the same way as the rat after intraperitoneal inoculation.

Three mice, inoculated subcutaneously, and one inoculated intraperitoneally, show general dissemination and enormous multiplication of bacilli, with little tissue change. In two other mice, inoculated subcutaneously, the bacilli are generally disseminated but in relatively scanty numbers.

Ponies 10 and 2.—Both animals were inoculated subcutaneously, the former with 100 mg. and the latter with 50 mg. In the former, the spleen shows small deposits of bacilli which have produced slight caseous change, but otherwise there is no evidence of dissemination beyond the nearest gland. In Pony 2 none of the organs examined (lungs, liver, kidneys, and spleen) show evidence of dissemination.

Pony 14, fed with 600 mg., shows no dissemination beyond the nearest glands.

Pony 32, inoculated intravenously with 10 mg., shows general dissemination of bacilli in small numbers, but no evidence of progressive disease.

ARTHUR EASTWOOD.

THE ACTION OF MAMMALIAN FINELY GRANULAR OXYPHIL LEUCOCYTES UPON AVIAN TUBERCLE BACILLI.

I.—Phagocytic action of the Calf's Finely Granular Oxyphil Leucocytes upon Avian Tubercle Bacilli.

METHOD OF INOCULATION—INTRAVENOUS.

Number of Calf.	Virus.	Dose of Bacilli.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
524	A VIII.	200 mg.	30	Lung	Very abundant.	31	1·8	Very small.
”	”	”	”	Liver††	*	0	0	0
”	”	”	”	Popliteal gland.††	*	Very few	—	—
544	A VIII.	10 mg.	33	Lung.	Very abundant.	47	2·4	Very small.
”	”	”	”	Liver	Very abundant.	29	1·5	Very small.
”	”	”	”	Portal gland	Enormous	42	2·0	Very small.
560	A X.	9 cc. of emulsion.	40	Lung	Very abundant.	120	6·0	Small.
”	”	”	”	Liver	Very abundant.	18	·7	Very small.
”	”	”	”	Popliteal gland ††	*	0	0	0
566	A X.	5 cc. of emulsion.	43†	Lung††	0	0	0	0
”	”	”	”	Liver††	0	0	0	0
”	”	”	”	Cervical gland.	Abundant	36	2·7	Small.
588	A X.	5 cc. of emulsion.	45	Lung	Very abundant.	102	4·4	Small.
”	”	”	”	Liver	Abundant (about 2,000).	13	·5	Very small.
”	”	”	”	Mediastinal gland.	Very abundant.	216	6·9	Rather small.
564	A X.	9 cc. of emulsion.	45	Lung	Very abundant.	10	·6	Very small.
”	”	”	”	Liver	Very abundant.	55	2·3	Very small.
”	”	”	”	Mediastinal gland.	Very abundant.	19	1·3	Very small.

* In each of the specimens marked thus, bacilli were very abundant, but leucocytes were rare, less than 1,000 being present in the entire area of the section.

† This animal was killed; all the others were fatally infected.

SUMMARY (omitting specimens marked thus ††).—In an area containing 13,000 leucocytes 738 bacilli were found within leucocytes, the percentage of leucocytes containing bacilli being 2·5.

THE ACTION OF MAMMALIAN FINELY GRANULAR OXYPHIL LEUCOCYTES UPON AVIAN TUBERCLE BACILLI—*continued.*

II.—Phagocytic action of the Swine's Finely Granular Oxyphil Leucocytes upon Avian Tubercle Bacilli.

Number of Pig.	Virus.	Mode of Infection.	Dose of Bacilli.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
248	A VIII.	Intravenous	50 mg.	47	Lung	*	A few	—	—
"	"	"	"	"	Liver	Very abundant.	191	7·5	Very small.
"	"	"	"	"	Spleen	Very abundant.	36	2·0	Very small.
"	"	"	"	"	Portal gland	Very abundant.	28	1·6	Very small.
104	P II.	Intraperitoneal	10 mg.	69	Liver	7	0	0	0
"	"	"	"	"	Mesenteric gland.	14	0	0	0
178	A VII.	Feeding	100 mg.	101	Lung	Very abundant.	Very large	46·5	Moderately high.
"	"	"	"	"	Liver	Enormous	Very large	49·2	Moderate.
"	"	"	"	"	Mesenteric gland.	Very abundant.	Very large	26·4	Moderate.
180	A VII.	Subcutaneous	50 mg.	105	Lung	448	0	0	0
"	"	"	"	"	Liver	263	0	0	0
"	"	"	"	"	Spleen	16	0	0	0
"	"	"	"	"	Mesenteric gland.	Abundant	315	19·2	Relatively small.
182	A VII.	Intraperitoneal	10 mg.	106	Lung	Very abundant.	0	0	0
"	"	"	"	"	Liver	Very abundant.	18	·7	Extremely small.
"	"	"	"	"	Spleen	Abundant	3	·2	Extremely small.
"	"	"	"	"	Mesenteric gland.	Enormous	Very large	45·0	Moderate.
236	A X.	Intravenous	Tissue emulsion.	120	Lung	*	A few	—	—
"	"	"	"	"	Liver	*	0	0	0
"	"	"	"	"	Spleen	*	0	0	0
"	"	"	"	"	Mesenteric gland.	*	A few	—	—

* In each of the specimens marked thus, bacilli were very abundant, but leucocytes were rare, less than 1,000 being present in the entire area of the section.

SUMMARY (omitting specimens marked thus *).—In an area containing 16,000 leucocytes the percentage of leucocytes containing bacilli was 12·4.

A NOTE ON THE COARSELY GRANULAR LEUCOCYTES IN SWINE.

In all the preceding counts of 1,000 leucocytes the coarsely granular cells are included. In the majority of cases the number of these is so small that it has not been found necessary to make a separate record of them. But in all cases where these cells are at all numerous they have been specially counted, with results shown in the following tables :—

(1.)

Coarsely Granular Leucocytes in Pigs infected with Mammalian Bacilli.

Number per 1,000 Leucocytes.	Tissue.	Number of Pig.
22	Liver	194
25	Submaxillary lymphatic gland.	20
28	Lung	20
61	Tracheal gland	92
100	Lung	186
158	Bronchial gland	186
168	Mesenteric gland	18
215	Spleen	186
230	Lung	49
268	Liver	186

No bacilli have been found in any coarsely granular leucocytes present in pigs infected with mammalian bacilli.

(2.)

Coarsely Granular Leucocytes in Swine infected with Avian Bacilli.

Number per 1,000 Leucocytes.	Number of these which contain Bacilli.	Tissue.	Number of Pig.
37	0	Lung	180
71	7	Mesenteric gland.	182
76	12	Liver	178
108	0	Mesenteric gland.	180
122	0	Liver	180
200	0	Spleen	180
217	0	Liver	248
255	20	Lung	178
280	0	Spleen	248
438	22	Mesenteric gland.	178
529	0	Portal gland	248

III.—Phagocytic action of the Rabbit's Finely Granular Oxyphil Leucocytes in early Stages of Infection with Avian Tubercle Bacilli.

(*a.*) EARLY STAGES OF INFECTION.

METHOD OF INOCULATION—INTRAVENOUS.

DOSE FOR EACH ANIMAL—1 MG. OF CULTURE OF VIRUS A X.

Number of Animal.	Duration of Experiment.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
1552	24 hours	Lung	3	0	0	0
„	„	Liver	56	3	0·3	5·3
„	„	Spleen	57	3	0·2	5·2
1553	3 days	Lung	63	0	0	0
„	„	Liver	66	2	0·2	3·0
„	„	Spleen	344	8	0·7	2·3
1554	5 days	Lung	93	0	0	0
,	„	Liver	149	4	0·4	2·6
„	„	Spleen	1,450	38	2·0	2·6
1793	6 days	Lung	51	5	·2	10·0
„	„	Liver	2,004	10	·6	·5
„	„	Spleen	1,656	56	3	3·4
1555	8 days	Lung	162	7	0·5	4·3
„	„	Liver	1,298	11	0·7	0·8
„	„	Spleen	2,750	74	3·1	2·6
1792	11 days	Lung	176	0	0	0
„	„	Liver*	Very abundant	36	2·7	Very small.
„	„	Spleen*	Very abundant	30	1·9	Very small.
1556	14 days	Lung	53	0	0	0
„	„	Liver*	Abundant colonies	4	0·4	Very small.
„	„	Spleen*	„ „	11	0·8	„

NOTE.—A lymphatic gland from each of the above animals has also been examined. In the 24 hours' and 3 days' specimens no bacilli were found in the areas containing 1,000 leucocytes; in the 5 days' specimen 7 bacilli were found in a similar area, in the 6 days' specimen there were 20 bacilli, in the 8 days' specimen 229 bacilli, and in the 11 and 14 days' specimens the bacilli had grown into numerous colonies; in no case were any bacilli found within leucocytes.

SUMMARY (omitting specimens marked thus *).—In an area containing 17,000 leucocytes 10,431 bacilli were found; 221 of these were within leucocytes, the percentage of leucocytes containing bacilli being ·7, and the percentage of bacilli within leucocytes 2·5.

Phagocytic action of the Rabbit's Finely Granular Oxyphil Leucocytes upon Avian Tubercle Bacilli.

(*b.*) LATER STAGES OF INFECTION.

Number of Animal.	Virus.	Dose of Bacilli.	Mode of Inoculation.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli.	Percentage of Bacilli within Leucocytes.
294	P II.	50 mg.	Intra-peritoneal.	13	Lung	286	6	·4	2·1
„	„	„	„	„	Liver	Very abundant.	13	·9	Very small.
„	„	„	„	„	Spleen	Very abundant.	47	2	Very small.

PHAGOCYTIC ACTION OF THE RABBIT'S FINELY GRANULAR OXYPHIL LEUCOCYTES UPON AVIAN TUBERCLE BACILLI—*continued.*

Number of Animal.	Virus.	Dose of Bacilli.	Mode of Inoculation.	Duration of Experiment in Days.	Organ Examined.	Number of Bacilli present in an area containing 1,000 Leucocytes.	Number of Bacilli contained within Leucocytes.	Percentage of Leucocytes containing Bacilli	Percentage of Bacilli within Leucocytes.
1791	A X.	1 mg.	Intravenous	18	Lung	Numerous (about 2,000).	0	0	0
"	"	"	"	"	Liver	Very abundant.	32	1·3	Very small.
"	"	"	"	"	Spleen	Enormous	22	·9	Very small.
292	P II.	1 mg.	Intra-peritoneal.	22	Lung	Very abundant.	11	·6	Very small.
"	"	"	"	"	Liver	Very abundant.	43	2·1	Very small.
"	"	"	"	"	Spleen	Very abundant.	79	3·4	Very small.
775	P XXVII.	1 mg.	Intravenous	25	Lung	Abundant	0	0	0
"	"	"	"	"	Liver†	*	0	0	0
"	"	"	"	"	Spleen†	Enormous	A few clumps.	Small	Very small.
326	P II.	5 mg.	Subcutaneous	69	Lung	137	0	0	0
"	"	"	"	"	Liver	13	0	0	0
"	"	"	"	"	Mesenteric gland.	Very abundant.	0	0	0
880	P XXI.	·001 mg.	Intravenous	140	Lung	Abundant	0	0	0
"	"	"	"	"	Liver	16	0	0	0
"	"	"	"	"	Spleen	116	0	0	0
957	A V.	10 mg.	Subcutaneous	168	Lung	Abundant	82	5·1	Rather small.
"	"	"	"	"	Liver	5	0	0	0
"	"	"	"	"	Spleen†	0	0	0	0

* In the specimen marked thus, bacilli were very abundant but leucocytes were rare, less than 1,000 being present in the entire area of the section.

SUMMARY (omitting specimens marked thus †).—In an area containing 18,000 leucocytes 335 bacilli were found within leucocytes, the percentage of leucocytes containing bacilli being 1.

IV.—Phagocytic action of the Goat's Finely Granular Oxyphil Leucocytes in Tissues Infected with Avian Bacilli.

In several of the specimens infected with avian tubercle bacilli, the bacilli have been too abundant to count or even to estimate with any approach to accuracy, and it has not been found possible in these cases to obtain a reliable differential stain for the leucocytes. But from the general appearance of the cells present in these tissues there is every indication that the action of the leucocytes upon the bacilli is very slight. This evidence is corroborated by five cases in which it has been found possible to make a count. In all five, bacilli are very abundant. The numbers of bacilli within leucocytes are, respectively, 0, 3, 3, 7, 45. The corresponding percentages of leucocytes containing bacilli are 0, ·2, ·2, ·3, 1·5.

Summary.

Leucocytes do not play an important part in attacking the avian tubercle bacillus. In some cases, notably in swine, the percentage of leucocytes found to contain bacilli is very high, but these are cases where the tissues are swarming with bacilli, which have made their way in large numbers into every sort of cell capable of ingesting bacilli.

PART III.

COMPARATIVE BACTERIOLOGY.

I.—ADDITIONAL OBSERVATIONS ON THE CULTURAL CHARACTERS OF BOVINE VIRUSES.

CULTURAL CHARACTERS OF INDIVIDUAL STRAINS.*

Since the publication of my last Report no new bovine viruses have been investigated, but additional experimental animals have been inoculated with the old viruses and the characters of the cultures obtained from them have been studied. As a convenient distinction from the term "virus," which is used to designate all the bacilli derived from a spontaneously infected bovine which formed a starting-point of the investigations, these cultures raised from particular animals are termed "strains."

I have examined 31 additional strains, and classified them on the same principle and by the same methods as in my previous Report. They are arranged, according to their abundance of growth, in Grades I, II, III, &c., which form an ascending scale, Grade I being the class containing the cultures which grow least well on the test media.

VIRUS—B IV.

(15.)

Strain.—CHIMPANZEE 22.

Material Received.—Primary culture, 54 days old, isolated from Chimpanzee 22.

Cultural Characters.—Growth was satisfactory on serum. On broth the surface was covered in five weeks with a moderately thick pellicle which afterwards exhibited some tendency to sink, but many thin patches remained floating. On glycerin-agar growth was poor; it consisted of a thin grey layer with slightly granular patches. On potato growth was scanty and non-pigmented.

CLASSIFICATION.

Amongst the average growths of Grade II.

(16.)

Strain.—DOG 116. (*Lung.*)

Material Received.—Two cultures were received, being the 7th and the 4th generations of cultures isolated from different parts of the lung of Dog 116 (pup).

Cultural Characters.—Both cultures grew rather poorly on serum. On broth one of them formed a thin grey pellicle which covered the surface in three weeks and exhibited no subsequent change; the other grew more slowly, producing a thin pellicle which was speckled with granular points. On glycerin-agar a scanty grey layer with a few raised colonies was produced. On potato the growths were very scanty and non-pigmented.

CLASSIFICATION.

Amongst the poorer growths of Grade II.

(17.)

Strain.—DOG 116. (*Mesenteric Gland.*)

Material Received.—Fourth generation, 7 days old, of culture isolated from the mesenteric gland of Dog 116 (pup).

Cultural Characters.—The growths on serum were rather poor. On broth the surface was about two-thirds covered in a month and then growth ceased; the pellicle was rather thick and granular. On glycerin-agar growth was scanty; some thin grey streaks appeared but very little was to be seen on the rest of the surface. On potato the growth was very scanty and non-pigmented.

CLASSIFICATION.

Amongst the poorer growths in Grade II.

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* Continued from previous Report. (App. to 2nd Int. Rep., Vol. IV.)

(18.)

Strain.—DOG 150.

Material Received.—Third generation, 8 days old, of culture isolated from Dog 150 (pup).

Cultural Characters.—Good growths were obtained on serum. On broth growth was very poor, no more than a few delicate islands being obtained; at the end of two months no more than half the surface was covered. On glycerin-agar the growth consisted of a grey haze, with a few denser patches. On potato the growth was very scanty and non-pigmented.

The bacilli exhibited only slight morphological divergence from the serum type.

CLASSIFICATION.

An average growth of Grade I.

(19.)

Strain.—G.P. 2528 from DOG 150.

Material Received.—Fourth generation, 8 days old, of culture isolated from G.P. 2528, which had been inoculated from Dog 150.

Cultural Characters.—Good growths were obtained on serum. On broth no more than a few delicate islands were formed; these had covered about half the surface at the end of two months. On glycerin-agar the growth consisted of a grey haze, amongst which were some colonies which commenced to develop during the second month, and finally became large and raised. On potato there was a very slight increase.

The bacilli exhibited only slight divergence from the serum type.

CLASSIFICATION.

Identical with the preceding strain.

(20.)

Strain.—RABBIT 1899.

Material Received.—Primary culture, 37 days old, isolated from Rabbit 1899.

Cultural Characters.—Growths on the test media were poor, and identical with those of the two preceding strains.

CLASSIFICATION.

Identical with the two preceding strains.

(21.)

Strain.—FOWL 394.

Material Received.—Primary culture, 81 days old, isolated from Fowl 394.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered in 14 days with a rather thin and unequal pellicle which subsequently showed a tendency to sink. On glycerin-agar the growth was unequal but moderately thick in places, with some raised colonies. On potato a rather scanty white growth was obtained.

CLASSIFICATION.

About the average of Grade III.

VIRUS—B VII.

(2.)

Strains.—FOWLS 442 and 444.

Material Received.—Primary culture, 56 days old, from Fowl 442, and third generation, 7 days old, of culture from Fowl 444.

Cultural Characters.—The two strains proved to be identical in characters. Good growths were obtained on serum. On broth the surfaces became covered in 14 days with rather thin and unequal pellicles, which soon afterwards became moist and sank. On glycerin-agar fairly thick layers were formed with a few fine wrinkles and warts. On potato the growths were pale and rather scanty.

CLASSIFICATION

Amongst the better growths of Grade III.

VIRUS—B VIII.

(2.)

Strain.—DOG 156.

Material Received.—Primary culture, 47 days old, isolated from Dog 156 (pup).

Cultural Characters.—Good growths were obtained on serum. On broth the surface had become covered with a thin opaque film at the end of the third week; the pellicle then became moist and sank. On glycerin-agar a thin grey layer with some denser streaks was obtained. On potato there was a very thin, white growth.

On broth, in a 24 days' film, the bacilli varied in length from 2 to 6 or 7 μ; on potato, in a 56 days film, they measured from 1 to 5 μ; and on glycerin-agar, in a 45 days' film, from 1 to 3 μ.

CLASSIFICATION.

An average growth of Grade II.

(3.)

Strain.—G.P. 2921 from DOG 142.

Material Received.—Second generation, 10 days old, of culture isolated from G.P. 2921, which was inoculated from Dog 142.

Cultural Characters.—Good growths were obtained on serum. On other media the growths resembled those of the preceding strain.

CLASSIFICATION.

An average growth of Grade II.

VIRUS—B IX.

(15.)

Strain.—CHIMPANZEE 18.

Material Received.—Seventh generation, 13 days old, of culture isolated from Chimpanzee 18.

Cultural Characters.—Growth on serum was satisfactory. On broth, in the earlier cultures, very little growth was obtained; a later culture covered rather more than half the surface with a somewhat thick pellicle which soon became moist and sank. On glycerin-agar growth was very poor; fairly large, discrete colonies developed out of particles of the inoculated material, but on the rest of the surface little more than a grey haze was formed; with later cultures a thin, uniform, grey layer was obtained. On potato the surface became slowly covered with small dry, white colonies.

On broth the bacilli were much longer and more irregularly stained than the serum type; on other media only a slight divergence from the serum type was noted.

CLASSIFICATION.

An average growth of Grade II.

(16.)

Strain.—DOG 130.

Material Received.—Fifth generation, 7 days old, of culture isolated from Dog 130.

Cultural Characters.—Growths on serum were satisfactory. On broth, a grey, rather dense pellicle was readily formed but became moist and sank before the surface was covered. On glycerin-agar growth was moderately rapid during the first month and then ceased; it appeared as grey, granular streaks which though fairly dense did not completely cover the surface. On potato, a moderate amount of grey growth was obtained.

On potato, in a 56 days' film, the bacilli varied in length from 1 to 5 or 6 μ and were generally curved and beaded. On other media there was only a slight divergence from the serum type, but irregularly stained forms were noted.

CLASSIFICATION.

Amongst the poorer growths in Grade III.

(17.)

Strain.—DOG 136.

Material Received.—Third generation, 17 days old, of culture isolated from Dog 136 (pup).

Cultural Characters.—Good growths were obtained on serum. On broth growth commenced rapidly but was of the moist type, and sank when the surface had become about half covered. On glycerin-agar the surface was covered, though not equally, with thick, raised patches which showed no sign of becoming warty or wrinkled. On potato the surface was fairly well covered with raised cream-coloured colonies.

On potato, in a 56 days' film, the bacilli varied in length from 1 to 6 μ; most of them were curved and about half were beaded. On glycerin-agar the bacilli only deviated slightly from the serum type.

CLASSIFICATION.

Amongst the better growths of Grade III.

(18.)

Strain.—G.P. 2935 from DOG 136.

Material Received.—Second generation, 10 days old, of culture isolated from G.P. 2935, which had been inoculated from Dog 136.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered in five weeks with a rather thin, opaque pellicle which showed no tendency to sink. On glycerin-agar and potato the growths were identical with those of the preceding strain.

CLASSIFICATION.

Amongst the better growths of Grade III.

(19.)

Strain.—MONKEY 126.

Material Received.—Second generation, 24 days old, of culture isolated from Monkey 126.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was readily covered with an opaque pellicle which rapidly became moist and sank. On glycerin-agar a moderately abundant granular growth was obtained. On potato the growth was moderately abundant and non-pigmented.

On potato, in a 56 days' film, the bacilli varied in length from ·75 to 5 μ, and a considerable number were irregularly stained. On other media the bacilli diverged only slightly from the serum type.

CLASSIFICATION.

Amongst the average growths of Grade III.

(20.)

Strain.—MONKEY 152.

Material Received.—Third generation, 12 days old, of culture isolated from Monkey 152.

Cultural Characters. — Growths on serum were satisfactory. On broth portions of a thick, moist pellicle were formed, but these sank before the surface was half covered. On glycerin-agar growth was very poor; with the exception of some fairly large, discrete colonies, the growth was very little denser than a grey haze. On potato the surface was covered with a thinnish layer, amongst which were several raised yellowish colonies.

CLASSIFICATION.

Amongst the poorer growths in Grade II.

(21.)

Strains.—FOWLS 430 and 432.

Material Received.—Primary cultures, 63 and 48 days old isolated, respectively from Fowls 430 and 432.

Cultural Characters.—Good growths were obtained on serum. On broth the surfaces became covered with moderately thick pellicles in a fortnight. The growths then became moist and sank. On glycerin-agar growth was rapid during the first fortnight, forming fairly thick and finely wrinkled layers. Subsequently very little increase took place. On potato moderately good, raised, white growths were obtained.

CLASSIFICATION.

Amongst the best growths of Grade III.

VIRUS—B XII.

(3.)

Strain.—DOG 160.

Material Received.—Fourth generation, 8 days old, of culture isolated from Dog 160 (pup).

Cultural Characters.—Good growths were obtained on serum. On broth no more than thin, scattered islands were obtained; these had covered about half the surface at the end of the second month. On glycerin-agar there was no more than a grey haze at the end of two months. On potato no growth was visible.

CLASSIFICATION.

Amongst the poorest growths of Grade I.

(4.)

Strain.—PONY 6.

Material Received.—Fifth generation, 68 days old, of culture isolated from Pony 6.

Cultural Characters.—Good growths were obtained on serum. On broth the cultures failed. On glycerin-agar a grey, rather scanty layer, but with some denser patches, was slowly formed. On potato there was a moderate amount of grey growth.

CLASSIFICATION.

Amongst the better growths of Grade I.

VIRUS—B XXII.

(3.)

Strain.—DOG 126.

Material Received.—Sixth generation, 7 days old, isolated from Dog 126 (pup).

Cultural Characters.—Growths on serum were satisfactory. On broth the surface was rapidly covered with a thin, grey, moist growth, most of which soon fell to the bottom, but a few denser patches remained floating. On glycerin-agar a dull grey layer covered the surface in a fortnight and gradually became moderately dense. On potato a scanty, non-pigmented growth was obtained.

CLASSIFICATION.

Amongst the average growths of Grade III.

VIRUS—B XXVI.

(2.)

Strain.—FOWL 84.

Material Received.—Primary culture, 37 days old, isolated from the lung of Fowl 84.

Cultural Characters.—Growths on serum were satisfactory. On broth the surface was covered in about three weeks with a moderately thick layer showing no tendency to sink; it remained floating at the end of the second month. On glycerin-agar growth was very scanty, amounting to not much more than a grey haze. On potato growth was rather poor, but some isolated, fairly large, cream-coloured colonies were formed.

CLASSIFICATION.

Amongst the average cultures of Grade II.

(3.)

Strain.—FOWL 450.

Material Received.—Third generation, seven days old, of culture isolated from Fowl 450.

Cultural Characters.—The growths on test media proved identical with those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

(4.)

Strain.—FOWL 516.

Material Received.—Primary culture, 40 days old, isolated from Fowl 516.

Cultural Characters.—Fairly good growths were obtained on serum, but on glycerin-agar the growth was poor, amounting to a little more than a grey haze.

CLASSIFICATION.

Amongst the better growths of Grade I.

(5.)

Strain.—G.P. 3331.

Material Received.—Third generation, 22 days old, of culture isolated from G.P. 3331.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar the growth was scanty and unequal, being in places no more than a grey haze.

CLASSIFICATION.

Amongst the poorer growths of Grade II.

(6.)

Strain.—PARROT 6.

Material Received.—Primary culture, 63 days old, isolated from Parrot 6.

Cultural Characters.—Good growths were obtained on serum. On broth some small, thin islands were formed. On glycerin-agar the layer on most of the surface was very thin, but a few large colonies were formed. On potato growth was scanty and non-pigmented.

CLASSIFICATION.

Amongst the better growths of Grade I.

(7.)

Strain.—PARROT 8.

Material Received.—Third generation, 13 days old, of culture isolated from Parrot 8.

Cultural Characters.—Good growths were obtained on serum. On broth no more than a few specks were seen. On glycerin-agar there was slightly more than a grey haze. On potato growth was very slight.

CLASSIFICATION.

Amongst the average growths of Grade I.

(8.)

Strains.—RABBITS 1903, 1904, and 1908.

Material Received.—Primary cultures 59, 54 and 44 days old, isolated respectively from Rabbits 1903, 1904 and 1908.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar the growths were all poor, amounting to slightly more than a grey haze.

CLASSIFICATION.

Amongst the better growths of Grade I.

CLASSIFICATION OF BOVINE STRAINS.

Grade.	Cultural Vigour Relative to Grade.	Virus.	Strain.
I.	Below average	B XII.	Dog 160.
,,	Average	B IV.	Dog 150.
,,	,,	B IV.	G.P. 2528 from Dog 150.
,,	,,	B IV.	Rabbit 1899.
,,	,,	B XXVI.	Parrot 8.
,,	Above average	B XII.	Pony 6.
,,	,,	B XXVI.	Fowl 516.
,,	,,	B XXVI.	Parrot 6.
,,	,,	B XXVI.	Rabbit 1903.
,,	,,	B XXVI.	Rabbit 1904.
,,	,,	B XXVI.	Rabbit 1908.
II.	Below average	B IV.	Dog 116 (Lung).
,,	,,	B IV.	Dog 116 (Mesenteric gland).
,,	,,	B IX.	Monkey 152.
,,	,,	B XXVI.	G.P. 3331.
,,	Average	B IV.	Chimpanzee 22.
,,	,,	B VIII.	Dog 156.
,,	,,	B VIII.	G.P. 2921 from Dog 142.
,,	,,	B IX.	Chimpanzee 18.
,,	,,	B XXVI.	Fowl 84.
,,	,,	B XXVI.	Fowl 450.
III.	Below average	B IX.	Dog 130.
,,	Average	B IV.	Fowl 394.
,,	,,	B IX.	Monkey 126.
,,	,,	B XXII.	Dog 126.
,,	Above average	B VII.	Fowl 442.
,,	,,	B VII.	Fowl 444.
,,	,,	B IX.	Dog 136.
,,	,,	B IX.	G.P. 2935 from Dog 136.
,,	,,	B IX.	Fowl 430.
,,	,,	B IX.	Fowl 432.

COMPARISON WITH PREVIOUS RESULTS.

My results, compared with those previously reported, are as follows :—

Virus—B IV.

No. of strains previously reported—14, *viz.*, 11 in Grade I, 1 in Grade II (Dog 116), 1 in Grade III (Dog 92), and 1 in Grade IV (Dog 18).

No. of new strains—7, *viz.*, 3 in Grade I (2 from Dog 150 and 1 from Rabbit 1899), 3 in Grade II (2 from Dog 116 and 1 from Chimpanzee 22), 1 in Grade III (Fowl 394).

Virus—B VII.

One strain previously reported, in Grade II.

Two new strains, both in Grade III (Fowls 442 and 444).

Virus—B VIII.

One strain previously reported, in Grade II.

Two new strains, both in Grade II (Dogs 142 and 156).

Virus—B IX.

No. of strains previously reported—14, *viz.*, 13 in Grade III and 1 in Grade IV (Dog 50).

No. of new strains—8, *viz.*, 2 in Grade II (Monkey 152 and Chimpanzee 18), and 6 in Grade III (3 from Dogs 126 and 136, 2 from Fowls 430 and 432, and 1 from Monkey 126).

Virus—B XII.

Two strains previously reported, 1 in Grade I and 1 in Grade II.

Two new strains, both in Grade I (Dog 160 and Pony 6).

Virus—B XXII.

Two strains previously reported, 1 in Grade I and 1 in Grade III (Dog 72).

One new strain, in Grade III (Dog 126).

Virus—B XXVI.

One strain previously reported, in Grade II.

Nine new strains, 6 in Grade I (Rabbits 1903, 1904, 1908, Parrots 6 and 8, Fowl 516), and 3 in Grade II (Fowls 84 and 450, G.P. 3331).

Results.

Compared with former results, the new strains do not exhibit any very important differences. Sometimes, though not invariably, a change in the direction of increased luxuriance has been observed after residence in the tissues of a dog. The results on birds, taken as a whole, show very little evidence of modification, though in some instances the cultures recovered from birds have grown rather better than previously. Their characters have always remained in strict conformity to the mammalian type.

II.—ADDITIONAL OBSERVATIONS ON THE CULTURAL CHARACTERS OF HUMAN VIRUSES.

CULTURAL CHARACTERS OF INDIVIDUAL STRAINS.

On two viruses, H 13. A.D. and H 53. D.H. I have reported previously; I now add observations on 15 additional strains belonging to these viruses. 109 strains belonging to 44 new viruses have also been investigated.

VIRUS—H 13. A.D.

(Human Bronchial Glands and Spleen—*continued.*)

(4.)

Strain.—CALF 1175. (*Mediastinal Gland.*)

Material Received.—Fifteenth generation, 26 days old, of culture isolated from a mediastinal gland of Calf 1175.

Cultural Characters.—Good growths were obtained on serum. On broth a thick pellicle was slowly formed; about three-quarters of the surface were covered in 6 weeks, and then growth ceased. On glycerin-agar an abundant, wrinkled and warty growth was very rapidly formed. On potato growth was rapid and abundant, yielding a brick-red pigment.

CLASSIFICATION.

Amongst the poorer growths of Grade V.

(5.)

Strain.—CALF 1177. (*Prescapular Gland.*)

Material Received.—Third generation, 26 days old, of culture isolated from the prescapular gland of Calf 1177.

Cultural Characters.—Growth was satisfactory on serum. On broth a thin pellicle, forming irregular patches, some translucent and some opaque, covered most of the surface in a month and then rapidly became moist and sank. On glycerin-agar no more than a grey haze was formed. On potato no growth was visible to the naked eye, but microscopically bacilli were found which, though resembling serum bacilli in type, were evidently new growths.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

(6.)

Strain.—CALF 1177. (*Spleen.*)

Material Received.—Third generation, 26 days old, of culture isolated from the spleen of Calf 1177.

Cultural Characters.—Satisfactory growths were obtained on serum. On broth growth was the same as with the preceding strain. On glycerin-agar a little more than a grey haze was formed. On potato there was a very scanty, non-pigmented layer.

On glycerin-agar some thickened, club shaped forms were observed.

CLASSIFICATION.

About the average growth of Grade I.

(7.)

Strain.—CALF 1237. (*Prescapular Gland.*)

Material Received.—Primary culture, 46 days old, isolated from the prescapular gland of Calf 1237.

Cultural Characters.—Satisfactory growths were obtained on serum. On broth the growth was the same as with the preceding strains. On glycerin-agar no more than a grey haze was formed. On potato there was a very scanty layer.

Some of the bacilli grown on potato were longer (4μ) than the serum type.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

(8.)

Strain.—CALF 1237. (*Bronchial Gland.*)

Material Received.—Primary culture, 46 days old, isolated from a bronchial gland of Calf 1237.

Cultural Characters.—Satisfactory growths were obtained on serum. On broth the growth was similar to that of the preceding strains. On glycerin-agar no more than a grey haze was formed. On potato no growth was discernible to the naked eye, but some evidence of growth was observed microscopically.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

(9.)

Strain.—CALF 1237. (*Portal Gland.*)

Material Received.—Primary culture, 46 days old, isolated from a portal gland of Calf 1237.

Cultural Characters.—Satisfactory growths were obtained on serum. On the other test media the growths were identical with those obtained with the last strain.

CLASSIFICATION.

Identical with Strain (8).

VIRUS—H 53. D.H. (*a*).

(Human Lupus—*continued.*)

(3.)

Strain.—CALF 1155. (*Prescapular Gland.*)

Material Received.—Primary culture, 21 days old, isolated from the prescapular gland of Calf 1155.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered in three weeks with a thin layer which afterwards became moist and sank; the surface then became covered again with a layer of the same type. On glycerin-agar growth progressed slowly, gradually becoming fairly dense, finely granular, and slightly wrinkled. On potato a moderate amount of cream-coloured growth was obtained.

CLASSIFICATION.

One of the best growths of Grade III.

(4.)

Strain.—CALF 1155. (*Lung.*)

Material Received.—Primary culture, 21 days old, isolated from the lung of Calf 1155.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered during the fourth week with a thin uniform layer which afterwards became moist and sank. On glycerin-agar, in the first culture, the growth was unequal, the surface being covered with a layer which was thin in some places and in others was denser and granular; but in later cultures the growth was distinctly better and equal to that of the preceding strain. On potato a thin white layer was formed; but in later cultures the growth was thicker and cream coloured.

CLASSIFICATION.

Amongst the better growths of Grade III.

(5.)

Strain.—CALF 1199.

Material Received.—Primary culture, 43 days old, isolated from the prescapular gland of Calf 1199.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered in a month; some patches were dense, but there was a tendency to become moist, and most of the pellicle sank during the fifth week. On glycerin-agar the surface was well covered by the end of the third week and a few minute wrinkles had appeared; but not much increase took place subsequently; at the end of two months there was a uniform, moderately thick layer with a few wrinkles. On potato a thin, cream-coloured layer was formed.

The bacilli on broth and glycerin-agar only diverged slightly from the serum type.

CLASSIFICATION.

Amongst the best growths of Grade III.

(6.)

Strain.—G.P. 2703 from CALF 1257.

Material Received.—Sixth generation, 10 days old, of culture isolated from G.P. 2703.

Cultural Characters.—Good growths were obtained on serum. On broth growth was slow; the pellicle was thin at first but gradually thickened; the surface did not become more than half covered. On glycerin-agar there was formed a grey, rather scanty layer of unequal density. On potato there was a white, scanty growth.

CLASSIFICATION.

About the average of Grade III.

(7.)

Strains.—CALVES 1295 (*Thoracic and Portal Glands*) and 1333.

Material Received.—Eighth generation 20 days old, ninth generation 20 days old, and eighth generation 20 days old, of cultures obtained respectively from Calf 1295 (thoracic gland), Calf 1295 (portal gland) and Calf 1333.

Cultural Characters.—On test media the cultural characters of all these three strains were identical with those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

(8.)

Strain.—PIG 85.

Material Received.—Primary culture, 28 days old, isolated from Pig 85.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became slowly covered with a moist layer, which was of unequal density and showed a tendency to sink. On glycerin-agar, scanty grey patches were slowly formed. On potato the growth was grey and rather scanty.

CLASSIFICATION.

Amongst the poorer growths of Grade III.

VIRUS—H 53. D.H. (*b*).

(Human Lupus.)

Strain.—G.P. 3216 from Second Original Material.

Material Received.—Sixth generation, 7 days old, of culture isolated from G.P. 3216, which had been inoculated with the second original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered with a thin pellicle in three weeks; there was no subsequent thickening. On glycerin-agar a uniform thin layer was obtained. On potato growth was white and very scanty.

CLASSIFICATION.

Amongst the poorer growths of Grade III.

VIRUS—H 60. W.B.

(1.)

(Human Bronchial Gland.)

(*a.*)

Strain.—G.P. 1825 from ORIGINAL MATERIAL.

Material Received.—Fifth generation, 13 days old, of culture isolated from G.P. 1825, which had been inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became nearly covered in a month with a rather thin, opaque layer; the growth showed some tendency to sink, but about half remained floating, though it showed no signs of thickening. On glycerin-agar the growth was rather slow and did not spread evenly over the surface; it became markedly granular but not dense or uniform. On potato there was a moderately thick layer which exhibited a brown pigmentation.

On broth, in a 19 days' film the bacilli showed only a slight deviation from the serum type. On glycerin-agar, in a 44 days' film, the bacilli varied in length from 1 to 6 μ, and occasionally forms as long as 7 or 8 μ were found; they were nearly all curved; the majority were uniformly stained, but some were beaded and some showed globular swellings; branched forms were present. On potato, in a 45 days' film, the bacilli varied in length from 2 to 8 μ; about half were curved and beaded.

CLASSIFICATION.

One of the best growths in Grade III.

(*b.*)

Strain.—CALF 1107. (*Prescapular Gland.*)

Material Received.—Primary culture, 23 days old, isolated from the prescapular gland of Calf 1107, which had been inoculated with the preceding strain.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered in less than four weeks with a thin, opaque, somewhat moist pellicle; part, but not the whole, of the pellicle afterwards sank. On glycerin-agar a thin, grey, uniformly distributed layer slowly developed; the growth never became dense. On potato a rather scanty, non-pigmented, layer was formed.

On potato the bacilli were long (1 to 6 μ), curved, and beaded; on broth and glycerin-agar they were much shorter, and were often irregularly stained.

CLASSIFICATION.

Intermediate between the poorest and the best growths of Grade III.

(*c.*)

Strain.—CALF 1107. (*Portal Gland.*)

Material Received.—Primary culture, 23 days old, isolated from the portal gland of Calf 1107, which had been inoculated with (*a*).

Cultural Characters.—Good growths were obtained on serum. On broth the greater part of the surface was covered in a month with an opaque moist pellicle; a week later nearly the whole of the growth had sunk. On glycerin-agar a dull grey layer slowly developed, but never became dense. On potato a rather scanty grey layer was obtained.

On potato the bacilli were shorter (1 to 4·5 μ) than with the preceding strain.

CLASSIFICATION.

Identical with the strain isolated from the prescapular gland of this calf.

(*d.*)

Strain.—RABBIT 1228.

Material Received.—Primary culture, 28 days old, isolated from Rabbit 1228, which had been inoculated with (*a*) after prolonged sub-culture on glycerinated media.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered in a month; the layer in some places was dense, but in others was much thinner; it showed no tendency to sink. On glycerin-agar the surface became covered with a fairly thick, finely granular, and finely-wrinkled layer, the rate of growth being moderately rapid. On potato growth was moderate in amount and cream-coloured.

On potato the bacilli were long (·75 to 5 μ), curved, and beaded; on other media they were shorter, rarely exceeding 3 μ, and on broth the staining was notably irregular.

CLASSIFICATION.

Amongst the poorest growths of Grade IV.

(2.)

(Human Lung.)

Strain.—G.P. 1827 from HUMAN LUNG.

Material Received.—Second generation, 23 days old, of culture isolated from G.P. 1827, which had been inoculated with the human lung.

Cultural Characters.—In the earlier generations growth on serum was poor; it improved on sub-culture. On broth the surface was half covered in three weeks with a grey, dense layer; after this period the pellicle showed some tendency to become moist and sink. On glycerin-agar a dense, wrinkled and granular layer was readily formed. On potato the growth was moderately good and exhibited a brown pigmentation.

On both glycerin-agar and potato the bacilli were long (1 to 6 μ), curved, and beaded. On broth they were much shorter (1 to 3 μ) and generally straight.

CLASSIFICATION.

Not quite equal to the best growths in Grade IV.

(3.)

(Human Mesenteric Gland.)

Strain.—G.P. 1829 from HUMAN MESENTERIC GLAND.

Material Received.—Second generation, 23 days old, of culture isolated from G.P. 1829, which had been inoculated with the human mesenteric glands.

Cultural Characters.—Good growths were obtained on serum. On broth growth was slow, not more than a third of the surface being covered in five weeks; the pellicle showed no sign of sinking, but gradually became denser and after a time a thinner growth shot out from the older pellicle. On glycerin-agar a thick, warty, and finely wrinkled layer rapidly formed. On potato a moderately dense layer of brown growth was produced.

On media other than serum the bacilli varied in length from 1 to 5 μ and were generally curved and, less frequently, beaded.

CLASSIFICATION.

Amongst the better growths of Grade IV.

(4.)

(Human Brain.)

Strain.—G.P. 1832 from HUMAN BRAIN.

Material Received.—Third generation, 13 days old, of culture isolated from G.P. 1832, which had been inoculated with the human brain.

Cultural Characters.—Fairly good growths were obtained on serum. On broth the surface was covered in three weeks with an opaque but rather thin pellicle which showed no tendency to sink. On glycerin-agar a thick, very wrinkled, and warty layer was produced in a month. On potato a fairly dense, brown, crumb-like layer was obtained.

On broth and glycerin-agar the bacilli were from 1 to 3·5 μ in length and generally stained uniformly. On potato many of the bacilli were much longer (6 to 8 μ) and were curved and regularly beaded.

CLASSIFICATION.

Amongst the better growths of Grade IV.

VIRUS—H 67. E.P.

(Pus from Human Scapular Abscess.)

(1.)

Strain.—G.P. 1914 FROM ORIGINAL MATERIAL.

Material Received.—Fourth generation, 18 days old, isolated from G.P. 1914, which had been inoculated with original material.

Cultural Characters.—Growth was rather poor on serum to begin with, but improved later. On broth the surface was covered in 3½ weeks with a rather thin but unequal pellicle containing several white, slightly raised colonies; there was no tendency for the pellicle to become moist. On glycerin-agar a dull grey layer spread over the surface and gradually became thick and finely wrinkled. On potato a rather poor growth, with some slight tendency to pigmentation was formed.

CLASSIFICATION.

Amongst the poorer growths in Grade IV.

(2.)

Strain.—CALF 1143.

Material Received.—Primary culture, 33 days old, isolated from Calf 1143.

Cultural Characters.—Good growths were obtained on serum. On broth growth was poor, the pellicle being thin and the surface not more than half covered at the end of two months. On glycerin-agar there was a uniform layer which was not very dense but exhibited some definite wrinkles. On potato growth was rather scanty and of a brownish colour.

CLASSIFICATION.

One of the poorest growths in Grade IV.

(3.)

Strains.—CALF 1329. (*Lung and Prescapular Gland.*)

Material Received.—Seventh generations, 81 and 107 days old, of cultures isolated respectively from the lung and prescapular gland of Calf 1329.

Cultural Characters.—On test media the growths of both strains were found to be identical with those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

VIRUS—H 68. R.B.

(Pus from Human Sacral Abscess.)

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Seventh generation, 14 days old, of culture isolated from original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered in a month with an opaque film of unequal density, which showed no tendency to sink. On glycerin-agar the surface was covered with a grey layer of unequal density which was warty and finely wrinkled. On potato a fairly thick, light brown layer was formed.

CLASSIFICATION.

Amongst the less abundant growths of Grade IV.

(2.)

Strain.—CALF 1167.

Material Received.—Fourth generation, 40 days old, of culture isolated from Calf 1167.

Cultural Characters.—Good growths were obtained on serum. On broth a fairly thick pellicle was obtained, which showed no tendency to sink. On glycerin-agar the growth was thick and covered with wrinkles and small warts. On potato a fairly abundant, bright yellow growth was obtained.

On potato the bacilli were from 1 to 6 μ in length, and were generally curved and beaded; on glycerin-agar and broth they were shorter (1 to 3·5 μ), and on broth straight and uniformly stained forms predominated.

CLASSIFICATION.

One of the better growths of Grade IV.

(3.)

Strain.—CALF 1361.

Material Received.—Seventh generation, 33 days old, of culture isolated from Calf 1361.

Cultural Characters.—On test media the cultural characters were found to be identical with those the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

(4.)

Strain.—COW 77. (*Prescapular Gland.*)

Material Received.—Primary culture, 44 days old, isolated from the prescapular gland of Cow 77.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar a thick and very wrinkled layer was obtained. On potato the growth was moderate in amount and deep brown in colour.

The bacilli on potato and glycerin-agar were from 1 to 5 μ in length and frequently curved and beaded.

CLASSIFICATION.

One of the average growths of Grade IV.

(5.)

Strain.—Cow 77. (*Milk.*)

Material Received.—Primary culture, 41 days old, isolated from the milk of Cow 77.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered in five weeks with a tough and fairly thick layer. On glycerin-agar the first cultures were poor, but when tested a short time afterwards an abundant and markedly wrinkled growth was obtained. On potato a moderate amount of yellow growth was obtained.

CLASSIFICATION.

One of the average growths of Grade IV.

VIRUS—H 69. F.K.

(1.)

(Human Mesenteric Gland.)

Strain.—G.P. 1926 from ORIGINAL MATERIAL.

Material Received.—Third generation, 10 days old, of culture isolated from G.P. 1926, which had been inoculated with original material.

Cultural Characters.—Growths on serum were satisfactory but not very good. On broth growth was slow; several small, rather dense islands appeared during the third week; at the end of two months these had extended by the production of a thinner growth at their periphery, and had covered two-thirds of the surface. On glycerin-agar growth commenced rather vigorously but ceased after the third week; the total yield was a grey layer containing some raised white colonies, but was only moderately dense and not wrinkled. On potato a scanty, yellow layer was obtained in three weeks, and then growth ceased.

The bacilli grown on media other than serum were rather longer but exhibited only a slight divergence from the serum type.

CLASSIFICATION.

Amongst the better growths in Grade III.

(2.)

(Human Meninges.)

Strain.—G.P. 1924 from ORIGINAL MATERIAL.

Material Received.—Ninth generation, 9 days old, of culture isolated from G.P. 1924, which had been inoculated with original material.

Cultural Characters.—Growth on serum was satisfactory, but not very good. On broth growth was obtained with some difficulty; small, dense islands appeared, but these were slow in extending, their behaviour in this respect resembling that of the preceding strain. The growths on glycerin-agar and potato were also identical with those of the preceding strain.

Like the preceding strain, the bacilli grown on media other than serum exhibited only a slight divergence from the serum type.

CLASSIFICATION.

Identical with the preceding strain.

VIRUS—H 70. N.W.

(1.)

(Human Cervical Gland.)

Strain.—G.P. 1933 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 12 days old, of culture isolated from G.P. 1933, which had been inoculated with original material.

Cultural Characters.—Growth on serum was fairly good. On broth the growth was slow and rather poor, but the pellicle was fairly thick and showed no tendency to sink. On glycerin-agar the growth was moderately dense but unequal; there were large patches which were thick and prominently warty. On potato, the growth was not very thick but exhibited a deep brown pigment.

On potato, in a 56 days' film, the bacilli varied in length from ·75 to 5 μ and a few longer forms (6 to 7 μ) were noted. The majority were curved and beading was common. Some of the bacilli were notably thick and many were slender. A branched form was found.

CLASSIFICATION.

Not quite equal to best growths in Grade IV.

(2.)

(Human Bronchial Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Seventh generation, 10 days old, of culture isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the growth was slow and did not completely cover the surface, but the pellicle was tough, in parts very dense, and showed no tendency to sink. On glycerin-agar a fairly dense, warty layer was formed. On potato the yield was moderate in amount and exhibited a deep brown pigment.

CLASSIFICATION.

About equal to the preceding strain.

(3.)

(Human Lung.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Fifth generation, 10 days old, of culture isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth growth commenced as discrete islands which gradually thickened but did not extend very rapidly; the pellicle as a whole was moderately thick and showed no tendency to become moist or sink. On glycerin-agar the growth was opaque, raised and granular in patches, but not uniformly dense; part of the surface was only thinly covered. On potato the growth was rather thin and exhibited a deep yellow pigment.

In a 14 days' film from a horse serum culture many of the bacilli were 3 μ in length or slightly longer and were more or less definitely beaded; but in a film from a 17 days' bovine serum culture the bacilli were of the usual type (·75 to 2 μ in length and uniformly stained).

CLASSIFICATION.

Amongst the poorer growths in Grade IV.

(4.)

(Human Mesenteric Gland.)

Strain.—G.P. 1928 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 10 days old, of culture isolated from G.P. 1928, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth a moderately dense pellicle was slowly formed; it was tough, and showed no tendency to sink. On glycerin-agar a fairly dense, granular, and warty layer was formed. On potato there was a moderate amount of growth which exhibited a deep brown pigment.

CLASSIFICATION.

About equal to strains (1) and (2).

VIRUS—H 71. L.V. (*a*).

(Human Lupus.)

Strain.—G.P. 1938 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 8 days old, of culture isolated from G.P. 1938, which had been inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth a tough, fairly thick, and warty pellicle was produced; the growth was slow and ceased before the surface was completely covered. On glycerin-agar a fairly thick, finely wrinkled layer was obtained. On potato there was a yellow, crumb-like growth.

CLASSIFICATION.

Amongst the poorer growths of Grade IV.

VIRUS—H 71. L.V. (*b*).

(Human Lupus.)

Strain.—G.P. 3021 from SECOND ORIGINAL MATERIAL.

Material Received.—Fifth generation, 40 days old, of culture isolated from G.P. 3021, which had been inoculated with the second original material.

Cultural Characters.—Good growths were obtained on serum. A thick and warty pellicle was formed on broth. On glycerin-agar the growth was copious and wrinkled, with a few warts. On potato a brown, fairly abundant growth was obtained.

CLASSIFICATION.

About the average of Grade V.

VIRUS—H 72. H.C.

(Pus from Abscess of Human Rib.)

Strain.—G.P. 1945 from ORIGINAL MATERIAL.

Material Received.—Fifth generation, 14 days old, of culture isolated from G.P. 1945, which had been inoculated with original material.

Cultural Characters.—On serum fairly good growths were obtained. On broth a pellicle of unequal density was formed, with some tendency to become moist and sink, though there were tougher and granular portions which remained floating. On glycerin-agar a dull grey layer with denser, granular patches was formed; it never became wrinkled or uniformly thick. On potato a rather scanty, yellowish brown growth was obtained.

On pure serum the bacilli exhibited more irregularity than usual; in a film from a 14 days' horse serum culture they varied in length from 1 to 3 μ, were often irregularly stained, and were frequently curved or beaded; but in a film from a 17 days' bovine serum culture they were rarely longer than 2 μ and the staining was uniform.

CLASSIFICATION.

Amongst the best growths in Grade III.

VIRUS—H 74. D.S.

(1.)

(Human Mesenteric Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Fourth generation, 7 days old, of culture isolated from the human mesenteric gland.

Cultural Characters.—Good growths were obtained on serum. On broth growth was obtained readily but was of unequal density, thick patches being interspersed amongst semi-transparent material; the pellicle showed a tendency to sink, but a few thick and somewhat granular patches remained floating. On glycerin-agar a thin grey layer was slowly formed; it became denser in patches and somewhat granular, but the total yield was rather scanty; later cultures, however, grew much better, being moderately abundant, granular, and wrinkled. On potato a scanty, non-pigmented growth was formed.

CLASSIFICATION.

Amongst the poorer growths of Grade IV.

(2.)

(Human Bronchial Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Fourth generation, 7 days old, of culture isolated from the human bronchial gland.

Cultural Characters.—Good growths were obtained on serum. On broth a thin pellicle, interspersed with much thicker and somewhat granular patches, was readily formed; most of the thin material sank after the end of the first month. On glycerin-agar a rather scanty grey layer with some denser areas was formed in the first cultures, but when tested again, much better growths were obtained. On potato there was a thin, non-pigmented layer.

CLASSIFICATION.

One of the poorest growths of Grade IV.

(3.)

(Human Spleen.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Fourth generation, 7 days old, of culture isolated from the human spleen.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered during the fourth week with a pellicle which was broken up into islands of unequal density, a few of which contained raised, warty points; the pellicle did not increase in thickness, but showed no tendency to sink. On glycerin-agar a rather scanty grey layer was formed containing some thicker, granular patches; later cultures yielded much better growths. On potato there was a scanty, non-pigmented layer.

CLASSIFICATION.

Amongst the poorer growths of Grade IV.

VIRUS—H 75. A.H.

(1.)

(Human Peritoneal Fluid.)

Strain.—G.P. 2018 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 25 days old, isolated from G.P. 2018, which had been inoculated with the human peritoneal fluid.

Cultural Characters.—Good growths were obtained on serum. On broth a uniform, fairly thick pellicle covered the surface at the end of three weeks, and showed no tendency to sink. On glycerin-agar the surface was covered with a fairly uniform, moderately dense layer, which gradually became wrinkled. On potato a rather thin, cream-coloured layer was formed.

CLASSIFICATION.

Amongst the poorer growths of Grade IV.

(2.)

(Human Lung.)

Strain.—G.P. 2459 from ORIGINAL MATERIAL.

Material Received.—Sixth generation, 13 days old, of culture isolated from G.P. 2459, which was inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth a thick layer covered the surface in three weeks and showed no subsequent tendency to sink. On glycerin-agar an abundant, warty and wrinkled layer was formed during the first fortnight. On potato the growth was abundant; the pigmentation was at first yellow and afterwards brown.

CLASSIFICATION.

Typical of Grade V.

VIRUS—H 76. G.M.

(Human Knee Joint.)

Strain.—G.P. 2888 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, five days old, of culture isolated from G.P. 2888, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth growth was poor; it consisted of thin islands which were tough and showed no tendency to sink, but failed to cover the surface. On glycerin-agar the growth was rapid, abundant, and prominently wrinkled. On potato a good, reddish brown growth was obtained.

CLASSIFICATION.

Amongst the average growths of Grade IV.

VIRUS—H 77. R.L.

(1.)

(Human Mesenteric Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 72 days old, isolated from the original material.

Cultural Characters.—Fairly good growths were obtained on serum. On broth the first two cultures failed; afterwards a slow and scanty growth was obtained, consisting of semi-translucent islands speckled with denser patches. On glycerin-agar, with the exception of a few dense colonies which very slowly formed, the growth was only slightly opaque and very thin. On potato growth was non-pigmented and very scanty.

On bovine serum, in an early culture, 15 days old, the bacilli were unusually long (·75 to 3·5 μ), but in later serum cultures they were shorter (·5 to 2 or 2·5 μ).

CLASSIFICATION.

Amongst the better growths of Grade I.

(2.)

(Human Liver.)

Strain.—G.P. 2102 from ORIGINAL MATERIAL.

Material Received.—Seventh generation, 14 days old, of culture isolated from G.P. 2102, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the cultures failed. On glycerin-agar there were a few isolated thickened patches, the rest of the surface being covered with little more than a grey haze. On potato growth was very scanty and non-pigmented.

In one of the cultures on bovine serum, 15 days old, the bacilli varied in length from ·75 to 4 μ, and occasional forms were found as long as 4·5 to 5·5 μ. The majority of the bacilli were uniformly stained; many were curved, and a few were either beaded or exhibited globular swellings.

CLASSIFICATION.

Amongst the growths of Grade I.; perhaps slightly poorer than the preceding strain.

VIRUS—H ·78. O.D.

(1.)

(Human Lung.)

Strain.—G.P. 2133 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 42 days old, isolated from G.P. 2133, which had been inoculated with the original material.

Cultural Characters.—The growths on serum were fairly good. On broth the surface became covered in about a month with a moderately thick layer showing no tendency to sink. On glycerin-agar an abundant, granular, and wrinkled layer was formed. On potato the growth was moderate in amount and coloured with a deep brown pigment.

On bovine serum, in a 15 days' film, the bacilli were rather longer than the average (·75 to 3 or 3·5 μ).

CLASSIFICATION.

Amongst the better growths of Grade IV.

(2.)

(Human Bronchial Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 69 days old, isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered in a month with a fairly thick, granular pellicle. On glycerin-agar a moderately thick, granular, and finely wrinkled growth was obtained. On potato there was a moderately abundant, brown growth.

CLASSIFICATION.

One of the average growths of Grade IV.

(3.)

(Human Meninges.)

Strain.—G.P. 2122 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 49 days old, isolated from G.P. 2122, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth a thick, tough pellicle was slowly formed, the surface being covered in seven weeks. On glycerin-agar the growth was thick, granular, and wrinkled. On potato the growth was moderately abundant and brown in colour.

On broth, glycerin-agar, and potato, the bacilli were rather shorter than the average, varying in length from ·75 to 3 μ.

CLASSIFICATION.

Amongst the poorer growths of Grade V.

VIRUS—H 79. J.N.

(Human Lung.)

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Second generation, 21 days old, of culture isolated from the human lung.

Cultural Characters. — Satisfactory growths were obtained on serum. On broth a very thick pellicle was formed and the surface was covered in a month. On glycerin-agar a thick warty layer was formed. On potato there was a yellowish brown, rather scanty growth.

The bacilli grown on serum, in a 14 days' film, were less regularly stained than usual.

CLASSIFICATION.

Amongst the poorer growths of Grade V.

(2.)

Strain.—G.P. 2154 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 10 days old, of culture isolated from G.P. 2154, which was inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth an opaque, fairly dense pellicle was formed without difficulty. On glycerin-agar there was a thick layer, thrown into prominent wrinkles. On potato the growth was cream coloured and rather scanty.

CLASSIFICATION.

Amongst the better growths of Grade IV.

(3.)

Strain.—RABBIT 1085 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 44 days old, isolated from Rabbit 1085.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar and potato the growths were identical with those of the preceding strain.

CLASSIFICATION.

Identical with strain (2).

(4.)

Strain.—DOG 31.

Material Received.—Primary culture, 31 days old, isolated from Dog 31.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar and potato the growths were identical with those of the preceding strain.

CLASSIFICATION.

Identical with strain (2).

(5.)

Strain.—DOG 33.

Material Received.—Primary culture, 31 days old, isolated from Dog 33.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became slowly covered with a tough, thin layer, which was irregular and semi-opaque. On glycerin-agar there was a moderately dense, granular, and wrinkled layer. On potato there was a moderate amount of brown growth.

The bacilli in a 14 days' serum film stained rather irregularly and a few forms as long as 4 μ were noted.

CLASSIFICATION.

Amongst the poorer growths of Grade IV.

(6.)

Strain.—CALF 1243.

Material Received.—Primary culture, 20 days old, isolated from Calf 1243.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered with a thick, homogeneous layer in a month. On glycerin-agar the growth was dense and thrown into prominent wrinkles, but not warty. On potato there was a fairly dense, brown growth.

CLASSIFICATION.

Amongst the poorer growths of Grade V.

(7.)

Strain.—CALF 1227.

Material Received.—Primary culture, 43 days old, isolated from the prescapular gland of Calf 1227.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was nearly covered during the second week; in the third week it was completely covered and the pellicle became uniformly thick. On glycerin-agar growth took place rapidly and became thick and very wrinkled, with a few warty elevations. On potato a moderately thick, yellow layer was formed.

CLASSIFICATION.

Amongst the growths of Grade V, but not equal to the best.

(8.)

Strains.—CALVES 1297, 1305, 1321 (*Mediastinal and Portal Glands*), and 1325.

Material Received.—Eighth generation, 33 days old; fifth generation, 41 days old; fifth generation, 33 days old; eighth generation, 33 days old; and seventh generation, 89 days old of cultures isolated respectively from Calf 1297, Calf 1305, Calf 1321 (mediastinal gland), Calf 1321 (portal gland), and Calf 1325.

Cultural Characters.—On test media the cultural characters of all these strains proved to be identical with those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

VIRUS—H 80. D.M.

(Human Lung.)

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 58 days old, isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth a homogeneous, moderately dense pellicle was slowly formed; it showed no tendency to sink. On glycerin-agar the surface was unequally covered, but on the whole was fairly dense and was studded with granular and warty colonies. On potato the surface was not uniformly covered, but there were numerous large, cream-coloured colonies.

CLASSIFICATION.

Amongst the poorer growths of Grade IV.

(2.)

Strain.—G.P. 2187 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 34 days old, of culture isolated from G.P. 2187, which was inoculated with original material.

Cultural Characters.—Growth was rather poor on serum for the first few generations, but afterwards became good. On broth the first culture was poor; a second culture covered the surface in a month with a thick, wrinkled pellicle. On glycerin-agar there was a thick, very wrinkled growth. On potato a moderate amount of raised, deep yellow growth was obtained.

On glycerin-agar and potato the bacilli were notably long, 1·5 to 7 μ.

CLASSIFICATION.

Amongst the average growths of Grade IV.

(3.)

Strain.—CALF 1245.

Material Received.—Second generation, 36 days old, of culture isolated from Calf 1245.

Cultural Characters.—Good growths were obtained on serum. On broth the first culture failed; a subsequent culture covered the surface in four weeks with a dense, wrinkled pellicle. On glycerin-agar there was formed a thick, very wrinkled layer, containing a few warty elevations. On potato a moderate amount of yellow growth was obtained.

CLASSIFICATION.

Amongst the poore growths of Grade V.

(4.)

Strain.—CALF 1265.

Material Received.—Primary culture, 37 days old, isolated from Calf 1265.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar a thick, wrinkled layer with some warty elevations, was formed. On potato there was a yellow, raised, crusty layer.

CLASSIFICATION.

Identical with the preceding strain.

(5.)

Strains.—CALVES 1279, 1281, 1369, and 1375.

Material Received.—Eighth generation, 33 days old; eighth generation, 41 days old; seventh generation, 33 days old; and eighth generation, 33 days old; of cultures isolated respectively from Calves 1279, 1281, 1369, and 1375.

Cultural Characters.—On test media the cultural characters of all these strains were almost identical with those of strain (1), the original material.

CLASSIFICATION.

Amongst the poorer growths of Grade IV.

(6.)

Strain.—DOG 19.

Material Received.—Primary culture, 38 days old, isolated from a coeliac gland of Dog 19.

Cultural Characters.—Good growths were obtained on serum. On broth a dense, wrinkled pellicle readily covered the surface. On glycerin-agar the growth was rapid, and became thick, with many warty elevations and some wrinkles. On potato there was a moderate amount of raised, brown growth.

CLASSIFICATION.

Identical with strains (3) and (4)

VIRUS—H 81. P.W.

(Human Lung.)

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 54 days old, isolated from the original material.

Cultural Characters.—Growth was satisfactory on serum. On broth the surface was covered in five weeks with a thick and warty layer. On glycerin-agar there was an abundant, warty, and very wrinkled layer. On potato there was a raised, yellow growth.

CLASSIFICATION.

Amongst the average growths of Grade V.

(2.)

Strain.—G.P. 2212 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 42 days old, isolated from G.P. 2212, which was inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar and potato the growths were identical with those of the preceding strain.

CLASSIFICATION.

Identical with strain (1).

(3.)

Strain.—CALF 1129.

Material Received.—Primary culture, 39 days old, isolated from Calf 1129.

Cultural Characters.—Good growths were obtained on serum. On broth, glycerin-agar, and potato the growths were identical with those obtained from strain (1).

CLASSIFICATION.

Identical with strain (1).

(4.)

Strain.—CALF 1145. (*Prescapular Gland.*)

Material Received.—Primary culture, 46 days old, isolated from the prescapular gland of Calf 1145.

Cultural Characters.—Good growths were obtained on serum. On broth, glycerin-agar, and potato the growths were identical with those obtained from strain (1).

CLASSIFICATION.

Identical with strain (1).

(5.)

Strain.—CALF 1145. (*Mediastinal Gland.*)

Material Received.—Primary culture. 46 days old, isolated from a mediastinal gland of Calf 1145.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar and potato the growths were identical with those obtained from the preceding strains.

CLASSIFICATION.

Identical with strain (1).

(6.)

Strains.—CALVES 1357 and 1399.

Material Received.—Eighth generation, 33 days old, and eighth generation, 20 days old, of cultures isolated respectively from Calves 1357 and 1399.

Cultural Characters.—On test media the cultural characters of the two strains proved identical with those of strain (1), the original material.

CLASSIFICATION.

Amongst the average growths of Grade V.

(7.)

Strain.—G.P. 2865 from HORSE 1.

Material Received.—Primary culture, 33 days old, isolated from G.P. 2865, which had been inoculated rom Horse 1.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became two-thirds covered in six weeks with a thick layer; no further extension took place. On glycerin-agar the first growth was poor; later cultures grew readily and became thick and very wrinkled. On potato an abundant, yellowish brown growth was obtained.

CLASSIFICATION.

Amongst the better growths of Grade IV.

(8.)

Strain.—PARROT 7.

Material Received.—Primary culture, 14 days old, isolated from Parrot 7.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became slowly covered with a thin layer which showed no tendency to sink. On glycerin-agar the surface became well covered with a very wrinkled layer. On potato the growth was fairly good, and brick-red in colour.

CLASSIFICATION.

Amongst the average growths of Grade IV.

VIRUS—H 82. G.F.

(1.)

(Human Mesenteric Gland.)

Strain.—G.P. 2239 from ORIGINAL MATERIAL.

Material Received.—Second generation, 15 days old, of culture isolated from G.P. 2239, which was inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth growth was very slow; a moderately thick pellicle was formed, and showed no tendency to sink, but the surface never became more than half covered. On glycerin-agar the growth was moderately thick and very wrinkled. On potato the growth was cream-coloured and rather scanty.

CLASSIFICATION.

Amongst the poorer growths of Grade IV.

(2.)

(Human Lung.)

Strain.—G.P. 2242 from ORIGINAL MATERIAL.

Material Received.—Sixth generation, 14 days old, of culture isolated from G.P. 2242, which was inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth a very thick pellicle slowly developed and finally covered the surface. On glycerin-agar there was a thick layer, finely wrinkled and studded with small warts. On potato there was a fairly abundant, cream-coloured growth.

CLASSIFICATION.

Amongst the better growths of Grade IV.

VIRUS—H 83. G.C.

(1.)

(Human Mesenteric Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Sixth generation, 14 days old, of culture isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth a very thick pellicle was formed, covering the surface in about five weeks. On glycerin-agar there was a thick, warty, and wrinkled growth. On potato there was a moderately thick, cream-coloured layer.

In a film from one of the serum cultures, 14 days old, some of the bacilli were beaded and exceptionally long (5 to 6 μ).

CLASSIFICATION.

One of the average growths in Grade V.

(2.)

(Human Bronchial Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Fifth generation, 14 days old, of culture isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar and potato the cultures were identical with those of the previous strain.

The bacilli grown on serum did not exhibit any exceptional forms.

CLASSIFICATION.

Identical with strain (1).

(3.)

(Human Meninges.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 19 days old, isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth, glycerin-agar, and potato the growths were identical with those of strain (1).

CLASSIFICATION.

Identical with strain (1).

VIRUS—H 84. M.S.

(Human Lupus.)

Strain.—G.P. 2277 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 28 days old, isolated from G.P. 2277, which was inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth many large thick islands appeared; from these a finer growth spread out and the surface then became well covered. On glycerin-agar a fairly dense and very wrinkled layer was formed. On potato there was a moderate amount of growth, coloured with a brownish red pigment.

CLASSIFICATION.

Amongst the best growths of Grade IV.

VIRUS—H 85. H.B.

(Human Lupus.)

(1.)

Strain.—G.P. 2363 from ORIGINAL MATERIAL.

Material Received.—Seventh generation, 13 days old, of culture isolated from G.P. 2363, which was inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth a thin, grey pellicle was readily formed, but rapidly sank; in a second culture, part of the pellicle remained floating until the end of the second month, and increased in thickness. On glycerin-agar there was a rather thin grey layer, studded with some denser colonies. On potato the surface was covered with a moderate amount of cream-coloured growth.

CLASSIFICATION.

Amongst the average growths of Grade III.

(2.)

Strain.—CHIMPANZEE 11. (*Mesenteric Gland.*)

Material Received.—Primary culture, 34 days old, isolated from a mesenteric gland of Chimpanzee 11.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered in a fortnight with a thin layer which then began to sink. On glycerin-agar an unequal layer, dense in patches, was formed. On potato there was a moderate amount of grey growth.

CLASSIFICATION.

Amongst the average growths of Grade III.

(3.)

Strain.—CHIMPANZEE 11. (*Spleen.*)

Material Received.—Primary culture, 34 days old, isolated from the spleen of Chimpanzee 11.

Cultural Characters.—On serum and on all the test media the growths were identical with those of the preceding strain.

CLASSIFICATION.

Amongst the average growths of Grade III.

(4.)

Strain.—MONKEY 121.

Material Received.—Primary culture, 41 days old, isolated from Monkey 121.

Cultural Characters.—On serum the first growths obtained were poor; better, but not very abundant yields were obtained after subculture for a few generations. On broth (three attempts) no more than a few minute islands were obtained. On glycerin-agar a very thin but definitely grey layer was formed. On potato a very scanty grey layer was slowly formed.

CLASSIFICATION.

Amongst the poorest growths of Grade II.

VIRUS—H 86. C.P.

(Human Meninges.)

Strain.—G.P. 2395 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 30 days old, isolated from G.P. 2395, which was inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered in a month with a uniform grey layer which was not very dense, and did not subsequently thicken; it showed no tendency to sink. On glycerin-agar a very wrinkled layer was formed. On potato there was a moderate amount of yellow growth.

CLASSIFICATION.

One of the average growths of Grade IV.

VIRUS—H 87. W.D.

(Human Suprarenal Capsule.)

Strain.—G.P. from ORIGINAL MATERIAL.

Material Received.—Primary culture, 35 days old, isolated from a guinea-pig inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth a tough pellicle was slowly formed. On glycerin-agar some of the earlier cultures appeared unusually moist; later cultures formed a typical dry, thick, wrinkled growth, with some warts. On potato a moderate amount of brownish yellow growth was formed.

CLASSIFICATION.

Amongst the better growths of Grade IV.

VIRUS—H 88. E.L.

(Human Mesenteric Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Fifth generation, 15 days old, of culture isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth growth commenced rapidly and covered the surface with a thin layer, but during the fifth week became moist and sank. On glycerin-agar the surface became uniformly covered with a thin, grey, opaque layer. On potato a scanty non-pigmented growth was formed.

CLASSIFICATION.

Amongst the average growths of Grade III.

VIRUS—H 89. W.I.

(1.)

(Human Mesenteric Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Seventh generation, 13 days old, of culture isolated from the original material.

Cultural Characters.—Satisfactory growths were obtained on serum. On broth four cultures were tried, but on three no more than a few scattered islands were obtained; the best of the four covered two-thirds of the surface at the end of two months with a very delicate layer. On glycerin-agar there was very little more than a grey haze. On potato a few isolated grey colonies were found.

On media other than serum the bacilli deviated only slightly from the serum type.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

(2.)

(Human Lung.)

Strain.—G.P. 2474 from ORIGINAL MATERIAL.

Material Received.—Twenty-fifth generation, 16 days old, of culture isolated from G.P. 2474, which had been inoculated with original material.

Cultural Characters.—The cultural characters on serum, broth, glycerin-agar, and potato resembled those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

VIRUS—H 90. I.P.

(1.)

(Human Retro-Peritoneal Gland.)

(*a*.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 46 days old, isolated from the original retro-peritoneal gland.

Cultural Characters.—Good growths were obtained on serum. On broth the growth was difficult to start, but ultimately a fairly dense layer, of unequal thickness but showing no tendency to sink, was formed. On glycerin-agar a thick, warty and wrinkled layer was readily formed. On potato there was a fairly good growth, exhibiting brown pigmentation.

On potato some of the bacilli were very long (6 to 8 μ).

CLASSIFICATION.

Amongst the better growths of Grade IV.

(*b*.)

Strains.—CALVES 1383 and 1471.

Material Received.—Sixth generations, 16 days old, of cultures isolated from Calves 1383 and 1471.

Cultural Characters.—The strains were found to be identical in cultural characters. Growth was fairly good on serum. On broth a rather thin, semi-opaque pellicle was slowly formed which readily became moist and sank. On glycerin-agar there was a thin, slightly opaque layer. On potato very little increase was obtained.

CLASSIFICATION.

Amongst the better growths of Grade II.

(2.)

(Human Mesenteric Gland.)

Strain.—G.P. 2522 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 27 days old, isolated from G.P. 2522, which was inoculated with the original mesenteric gland.

Cultural Characters.—Good growths were obtained on serum. On broth growth was rather difficult to start and, when obtained, exhibited the same characters as with the last strain. On glycerin-agar the growth was rapid and thick, but less prominently warty and wrinkled than with the last strain. On potato there was a good growth, showing a bright yellow pigmentation.

On broth the bacilli only diverged slightly from the serum type. On glycerin-agar and on potato they varied in length from 1 to 6 μ, and were generally curved and beaded.

CLASSIFICATION.

Amongst the average growths of Grade IV.

VIRUS—H 91. H.S.

(Human Lupus.)

Strain.—G.P. 2590 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 45 days old, isolated from G.P. 2590, which was inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth an irregular, thin, moist pellicle was rapidly formed and sank before the surface became completely covered. On glycerin-agar there was a scanty grey layer with a few warty points. On potato scanty, grey colonies appeared.

CLASSIFICATION.

Amongst the poorer growths of Grade III.

VIRUS—H 92. D.N.

(Human Lupus.)

Strains.—G.Ps. 2592 and 2593 from ORIGINAL MATERIAL.

Material Received.—Primary cultures, each 45 days old, from G.Ps. 2592 and 2593, which were inoculated with original material.

Cultural Characters.—The two cultures proved to be identical in characters. Good growths were obtained on serum. On broth thick, wrinkled pellicles were formed and covered the surface in about five weeks. On glycerin-agar the growths were abundant, warty, and wrinkled. On potato raised brownish, yellow, crumb-like growths were produced.

CLASSIFICATION.

Typical of Grade V.

VIRUS—H 93. C.D.

(Human Lung and Acne Spot.)

Strains.—G.Ps. 2609 and 2611 from ORIGINAL MATERIAL.

Material Received.—Seventh generations, 10 days old, of cultures isolated from G.Ps. 2609 and 2611, which were inoculated with original material, the former with lung and the latter with material from an acne spot.

Cultural Characters.—The two cultures proved to be identical in characters. Good growths were obtained on serum. On broth moderately thick and tough pellicles were slowly formed; they showed no sign of sinking but gradually covered the surface and spread on to the glass. On glycerin-agar the surfaces were well covered with a thick layer which gradually became markedly wrinkled. On potato moderate amounts of yellowish brown growths were formed.

CLASSIFICATION.

Typical of Grade IV.

VIRUS—H 98. B.R.

(Human Cerebro-Spinal Fluid.)

Strain.—G.P. 2878 from ORIGINAL MATERIAL.

Material Received.—Sixth generation, 13 days old, of culture isolated from G.P. 2878, which had been inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth thin, small islands were formed; they covered about three-fourths of the surface at the end of three weeks, but no further development took place. On glycerin-agar growth was rapid and formed a very wrinkled but not warty layer. On potato there was a raised, yellowish-brown growth.

CLASSIFICATION.

Amongst the average growths of Grade IV.

VIRUS—H 99. L.K.

(Human Lupus.)

(1.)

Strain.—G.P. 2927 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 13 days old, isolated from G.P. 2927, which had been inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became gradually covered with a very thin layer, which showed no signs either of thickening or of sinking. On glycerin-agar a thick, very prominently wrinkled layer was formed. On potato growth was abundant and reddish-brown in colour.

CLASSIFICATION.

Amongst the better growths of Grade IV.

(2.)

Strain.—G.P. 2928 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 13 days old, isolated from G.P. 2928, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered in a month with a very thick, wrinkled pellicle. On glycerin-agar the first culture was rather scanty, but a subsequent culture was abundant, very warty, and wrinkled. On potato the growth was good, with a yellowish-red pigmentation.

CLASSIFICATION.

Amongst the average growths of Grade V.

VIRUS—H 100. R.S.

(Human Lupus.)

Strain.—G.P. 2929 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 48 days old, isolated from original material.

Cultural Characters.—The growth was at first poor on serum, but improved in later generations. On broth (3 attempts) no more than small, scattered, thin islands were obtained. On glycerin-agar there was a grey layer, thin on the whole, but sprinkled with many raised colonies. On potato a scanty grey layer was formed.

CLASSIFICATION.

Amongst the best growths of Grade II.

VIRUS—H 101. E.G.

(Human Lupus.)

Strain.—G.P. 2932 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 33 days old, isolated from G.P. 2932, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became slowly covered with a thin layer which showed no tendency to sink. On glycerin-agar the surface was covered with a fairly dense grey layer, showing no tendency to become wrinkled or warty. On potato a moderate amount of brown growth was obtained.

CLASSIFICATION.

Amongst the best growths of Grade III.

VIRUS—H 102. N.H.

(Human Lupus.)

Strain.—G.P. 2934 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 41 days old, isolated from G.P. 2934, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became about three-quarters covered with a moderately dense layer showing no tendency to sink. On glycerin-agar a dense, wrinkled layer was formed. On potato growth was abundant and showed a brown pigmentation.

CLASSIFICATION.

Amongst the better growths of Grade IV.

VIRUS—H 103. N.S.

(Human Lupus.)

Strain.—G.P. 2938 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 48 days old, isolated from G.P. 2938, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became slowly covered with a homogeneous, rather thin layer, which did not thicken but showed no tendency to sink. On glycerin-agar the growth was dense and wrinkled and showed some small warts. On potato a moderate amount of brick-red growth was obtained.

The bacilli were found to be unusually long, those grown on serum (14 days) measuring from 1·5 to 5 μ, on broth (22 days) 2·5 to 8 μ, on glycerin-agar (42 days) 2 to 7 or 8 μ, on potato (2 months) 1·5 to 8 μ.

CLASSIFICATION.

Amongst the average growths of Grade IV.

VIRUS—H 104. E.R.

(Human Lung.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 37 days old, isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered in five weeks with a fairly thick, tough layer. On glycerin-agar a thick, wrinkled, and warty growth was obtained. On potato there was an abundant, reddish-brown growth.

CLASSIFICATION.

Amongst the average growths of Grade V.

VIRUS—H 105. G.S.

(Human Lupus.)

Strain.—G.P. 2850 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 40 days old, isolated from G.P. 2850, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth no more than a few small islands were obtained. On glycerin-agar there was a scanty, unequally distributed, greyish layer. On potato a thin grey layer was formed.

CLASSIFICATION.

Amongst the average growths of Grade II.

VIRUS—H 106. K.R.

(Human Lupus.)

Strain.—G.P. 2952 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 38 days old, isolated from G.P. 2952, which had been inoculated with the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface became nearly covered in two months with a thin pellicle which showed no tendency to sink. On glycerin-agar there was a grey, slightly wrinkled, but not uniformly thick layer. On potato a fair amount of reddish-brown growth was obtained.

CLASSIFICATION.

Amongst the poorer growths of Grade IV.

VIRUS—H 107. H.H.

(Human Lupus.)

Strain.—G.P. 2955 from ORIGINAL MATERIAL.

Material Received—Primary culture, 36 days old, isolated from G.P. 2955, which had been inoculated with the original material.

Cultural Characters.—Growths on serum were at first poor, but in later generations improved. On broth no more than thin, scattered islands were obtained. On glycerin-agar the growth was moderately thick, with no wrinkles or warts. On potato a scanty grey layer was obtained.

CLASSIFICATION.

Amongst the average growths of Grade III.

VIRUS—H 108. H.R.

(Human Lupus.)

Strain.—G.P. 2957 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 36 days old, isolated from G.P. 2957, which had been inoculated with the original material.

Cultural Characters.—The first cultures on serum were poor; later generations grew better. On broth the greater part of the surface became covered with discrete, thin islands. On glycerin-agar a grey, rather scanty layer was obtained. On potato discrete, grey colonies were formed.

CLASSIFICATION.

Amongst the poorer growths of Grade III.

VIRUS—H 109. M.W.

(Human Lupus.)

Strain.—G.P. 3002 from ORIGINAL MATERIAL.

Material Received.—Seventh generation, 20 days old, of culture isolated from G.P. 3002, which had been inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth a thick, tough pellicle was slowly formed, but failed to cover more than three-fourths of the surface. On glycerin-agar a thick, markedly wrinkled growth was obtained. On potato there was a fairly good, light yellow growth.

CLASSIFICATION.

Amongst the best growths of Grade IV.

VIRUS—H 110. J.B. (*a*).

(Human Lupus.)

Strain.—G.P. 3007 from ORIGINAL MATERIAL.

Material Received.—Tenth generation, 8 days old, of culture isolated from G.P. 3007, which had been inoculated with original material.

Cultural Characters.—The growths on serum were poor at first but became good after subculture. On broth growth was poor, consisting of thin, scanty islands. On glycerin-agar a very thin layer interspersed with some thicker patches was slowly formed. On potato there were a few small, cream-coloured patches.

CLASSIFICATION.

Amongst the average growths of Grade II.

VIRUS—H 110. J.B. (*b*)

(Human Lupus.)

Strain.—G.P. 3289 from SECOND ORIGINAL MATERIAL.

Material Received.—Primary culture, 20 days old, isolated from G.P. 3289, which had been inoculated with the second original material.

Cultural Characters.—Growth on serum was poor at first, but afterwards became good. On other media the cultural characters were identical with those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

VIRUS—H 111. S.E.

(Human Lupus.)

Strain.—G.P. 3009 from ORIGINAL MATERIAL.

Material Received.—Eleventh generation, 20 days old, of culture isolated from G.P. 3009, which had been inoculated with original material.

Cultural Characters.—Growth on serum was poor at first, but became good on subculture. On broth a fairly thick pellicle covered two-thirds of the surface in three weeks, and then began to sink. On glycerin-agar the surface became slowly covered with an unequal growth exhibiting some dense patches. On potato a grey, rather scanty growth was formed.

CLASSIFICATION.

Amongst the average growths of Grade III.

VIRUS—H 112. B.B.

(Human Lupus.)

Strain.—G.P. 3042 from ORIGINAL MATERIAL.

Material Received.—Sixth generation, 20 days old, of culture isolated from G.P. 3042, which had been inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth a thick wrinkled pellicle was gradually formed. On glycerin-agar the growth was rapid, wrinkled, and somewhat warty. On potato there was a fairly abundant, raised brown growth

CLASSIFICATION.

Amongst the poorer growths of Grade V.

VIRUS—H 114. A.U.

(Human Lupus.)

Strain.—G.P. 3046 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 20 days old, isolated from G.P. 3046, which had been inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth a thick pellicle was formed in three weeks and showed no tendency to sink. On glycerin-agar a thick, very wrinkled, and somewhat warty layer was readily formed. On potato there was a raised, fairly abundant, yellow growth.

CLASSIFICATION.

About the average of Grade V.

VIRUS—H 115. N.G.

(Human Cerebro-Spinal Fluid.)

Strains.—G.Ps. 3086 and 3087 from ORIGINAL MATERIAL.

Material Received.—Primary cultures, each 44 days old, from G.Ps. 3086 and 3087, which were inoculated with original material.

Cultural Characters.—Both strains proved identical. Good growths were obtained on serum. On broth fairly thick pellicles, with no tendency to sink, were formed rather slowly. On glycerin-agar thick, very wrinkled growths were readily formed. On potato there were obtained fairly abundant, light yellow growths.

CLASSIFICATION.

Amongst the best growths of Grade IV.

CLASSIFICATION OF HUMAN STRAINS.

Grade.	Cultural Vigour Relative to Grade.	Strain.	Virus.	Original Human Material.
I.	Below average	Calf 1177 (Prescapular Gland) ...	H 13. A.D.	Bronchial glands and spleen.
,,	,,	Calf 1237 (Prescapular Gland) ...	,,	,, ,,
,,	,,	,, (Bronchial Gland) ...	,,	,, ,,
,,	,,	,, (Portal Gland)	,,	,, ,,
,,	,,	Original Material	H 89. W.I.	Mesenteric gland.
,,	,,	G.P. 2474 from Original Material	,,	Lung.
,,	Average	Calf 1177 (Spleen)	H 13. A.D.	Bronchial glands and spleen.
,,	,,	Original Material	H 77. R.L.	Liver.
,,	Above average	Original Material	,,	Mesenteric gland.
II.	Below average	Monkey 121	H 85. H.B.	Lupus.
,,	Average	G.P. 2850 from Original Material	H 105. G.S.	Lupus.
,,	,,	G.P. 3007 from Original Material	H 110. J.B. (*a*)	Lupus.
,,	,,	G.P. 3289 from Original Material	,, (*b*)	,,
,,	Above average	Calf 1383	H 90. I.P.	Retro-peritoneal gland.
,,	,,	Calf 1471	,,	,, ,,
,,	,,	G.P. 2929 from Original Material	H 100. R.S.	Lupus.
III.	Below average	Pig 85	H 53. D.H. (*a*)	Lupus.
,,	,,	G.P. 3216 from Second Original Material.	,, (*b*)	,,
,,	,,	G.P. 2590 from Original Material	H 91. H.S.	Lupus.
,,	,,	G.P. 2957 from Original Material	H 108. H.R.	Lupus.
,,	Average	G.P. 2703 from Calf 1257	H 53. D.H. (*a*)	Lupus.
,,	,,	Calf 1295 (Thoracic Gland) ...	,, (*a*)	,,
,,	,,	,, (Portal Gland)	,, (*a*)	,,
,,	,,	Calf 1333	,, (*a*)	,,
,,	,,	Calf 1107 (Prescapular Gland) ...	H 60. W.B.	Bronchial glands.
,,	,,	,, (Portal Gland)	,,	,,
,,	,,	G.P. 2363 from Original Material	H 85. H.B.	Lupus.
,,	,,	Chimpanzee 11 (Spleen)	,,	,,
,,	,,	,, 11 (Mesenteric Gland)	,,	,,
,,	,,	Original Material	H 88. E.L.	Mesenteric gland.
,,	,,	G.P. 2955 from Original Material	H 107. H.H.	Lupus.
,,	,,	G.P. 3009 from Original Material	H 111. S.E.	Lupus.
,,	Above average	Calf 1155 (Prescapular Gland) ...	H 53. D.H. (*a*)	Lupus.
,,	,,	,, (Lung)	,, (*a*)	,,
,,	,,	Calf 1199	,, (*a*)	,,
,,	,,	G.P. 1825 from Original Material	H 60. W.B.	Bronchial gland.

CLASSIFICATION OF HUMAN STRAINS—*continued.*

Grade.	Cultural Vigour Relative to Grade.	Strain.	Virus.	Original Human Material
III.	Above average	G.P. 1926 from Original Material	H 69. F.K.	Mesenteric gland.
„	„	G.P. 1924 from Original Material	„	Meninges of brain.
„	„	G.P. 1945 from Original Material	H 72. H.C.	Abscess of rib.
„	„	G.P. 2932 from Original Material	H 101. E.G.	Lupus.
IV.	Below average	Rabbit 1228	H 60. W.B.	Bronchial gland.
„	„	G.P. 1914 from Original Material	H 67. E.P.	Scapular abscess.
„	„	Calf 1143	„	„
„	„	Calf 1329 (Lung)	„	„
„	„	„ (Prescapular Gland) ...	„	„
„	„	Original Material	H 68. R.B.	Sacral abscess.
„	„	Original Material	H 70. N.W.	Lung.
„	„	G.P. 1938 from Original Material	H 71. L.V. (*a*)	Lupus.
„	„	Original Material	H 74. D.S.	Mesenteric gland.
„	„	Original Material	„	Bronchial gland.
„	„	Original Material	„	Spleen.
„	„	G.P. 2018 from Original Material	H 75. A.H.	Peritoneal fluid.
„	„	Dog 33	H 79. J.N.	Lung.
„	„	Original Material	H 80. D.M.	Lung.
„	„	Calf 1279	„	„
„	„	Calf 1281	„	„
„	„	Calf 1369	„	„
„	„	Calf 1375	„	„
„	„	G.P. 2239 from Original Material	H 82. G.F.	Mesenteric gland.
„	„	G.P. 2952 from Original Material	H 106. K.R.	Lupus.
„	Average	G.P. 1827 from Original Material	H 60. W.B.	Lung.
„	„	Cow 77 (Prescapular Gland) ...	H 68. R.B.	Sacral abscess.
„	„	„ (Milk)	„	„
„	„	G.P. 1933 from Original Material	H 70. N.W.	Cervical gland.
„	„	Original Material	„	Bronchial glands.
„	„	G.P. 1928 from Original Material	„	Mesenteric glands.
„	„	G.P. 2888 from Original Material	H 76. G.M.	Knee joint.
„	„	Original Material	H 78. O.D.	Bronchial gland.
„	„	G.P. 2187 from Original Material	H 80. D.M.	Lung.
„	„	Parrot 7	H 81. P.W.	Lung.
„	„	G.P. 2395 from Original Material	H 86. C.P.	Meninges of brain.
„	„	G.P. 2522 from Original Material	H 90. I.P.	Mesenteric gland.
„	„	G.P. 2609 from Original Material	H 93. C.D.	Lung.
„	„	G.P. 2611 from Original Material	„	Acne spot.
„	„	G.P. 2878 from Original Material	H 98. B.R.	Cerebro-spinal fluid.
„	„	G.P. 2938 from Original Material	H 103. N.S.	Lupus.
„	Above average	G.P. 1829 from Original Material	H 60. W.B.	Mesenteric gland.
„	„	G.P. 1832 from Original Material	„	Brain.
„	„	Calf 1167	H 68. R.B.	Sacral abscess.
„	„	Calf 1361	„	„
„	„	G.P. 2133 from Original Material	H 78. O.D.	Lung.

CLASSIFICATION OF HUMAN STRAINS—*continued.*

Grade.	Cultural Vigour Relative to Grade.	Strain.	Virus.	Original Human Material.
IV.	Above average.	G.P. 2154 from Original Material	H 79. J.N.	Lung.
"	"	Rabbit 1085 from Original Material	"	"
"	"	Dog 31	"	"
"	"	G.P. 2865 from Horse 1	H 81. P.W.	Lung.
"	"	G.P. 2239 from Original Material	H 82. G.F.	Lung.
"	"	G.P. 2277 from Original Material	H 84. M.S.	Lupus.
"	"	G.P. from Original Material ...	H 87. W.D.	Suprarenal capsule.
"	"	Original Material	H 90. I.P.	Retro-peritoneal glands.
"	"	G.P. 2927 from Original Material	H 99. L.K.	Lupus.
"	"	G.P. 2934 from Original Material	H 102. N.H.	Lupus.
"	"	G.P. 3002 from Original Material	H 109. M.W.	Lupus.
"	"	G.P. 3086 from Original Material	H 115. N.G.	Cerebro-spinal fluid.
"	"	G.P. 3087 from Original Material	"	" "
V.	Below average	Calf 1175 (Mediastinal Gland) ...	H 13. A.D.	Bronchial glands and spleen.
"	"	G.P. 2122 from Original Material	H 78. O.D.	Meninges of brain.
"	"	Original Material	H 79. J.N.	Lung.
"	"	Calf 1243	"	"
"	"	Calf 1245	H 80. D.M.	Lung.
"	"	Calf 1265	"	"
"	"	Dog 19	"	"
"	"	G.P. 3042 from Original Material	H 112. B.B.	Lupus.
"	Average	G.P. 3021 from Original Material	H 71. L.V.(*b*)	Lupus.
"	"	G.P. 2459 from Original Material	H 75. A.H.	Lung.
"	"	Calf 1227	H 79. J.N.	Lung.
"	"	Calf 1297	"	"
"	"	Calf 1305	"	"
"	"	Calf 1321 (Mediastinal Gland) ...	"	"
"	"	" (Portal Gland)	"	"
"	"	Calf 1325	"	"
"	"	Original Material	H 81. P.W.	Lung.
"	"	G.P. 2212 from Original Material	"	"
"	"	Calf 1129	"	"
"	"	Calf 1145 (Prescapular Gland) ...	"	"
"	"	" (Mediastinal Gland) ...	"	"
"	"	Calf 1357	"	"
"	"	Calf 1399	"	"
"	"	Original Material	H 83. G.C.	Mesenteric gland.
"	"	Original Material	"	Bronchial gland.
"	"	Original Material	"	Meninges of brain.
"	"	G.Ps. 2592–3 from Original Material.	H 92. D.N.	Lupus.
"	"	G.P. 2928 from Original Material	H 99. L.K.	Lupus.
"	"	Original Material	H 104. E.R.	Lung.
"	"	G.P. 3046 from Original Material	H 114. A.U.	Lupus

SUMMARY OF RESULTS.

(1) Additional Strains of Old Viruses.

Virus—H 13. A.D.

Three strains previously reported, 1 in Grade IV (Calf 301) and 2 in Grade I (Rat 15 from Calf 301 and Calf 321 from Calf 301).

Six new strains, 1 in Grade V (Calf 1175 from Calf 301) and 5 in Grade I (2 strains of Calf 1177 from Calf 301 and 3 strains of Calf 1237 from Calf 1175).

Virus—H 53. D.H.

Two strains previously reported, both in Grade III.

Nine new strains, all in Grade III, 8 derived through various animals from the same original human material (*a*), and 1 derived from the second original material (*b*) obtained from the same subject.

Comments.

These results confirm those previously obtained. In the case of H 13. A.D. it has again been shown that cultures of very much lower luxuriance may, by animal passage, be obtained from the culture derived from Calf 301. The classification of H 53. D.H. has been confirmed by 8 additional strains derived, ultimately, from the same original material, and by a strain derived from tissue removed $3\frac{8}{12}$ years subsequently from the same human subject.

(2) New Viruses.

In 22 out of the 44 new viruses it was not considered necessary to investigate more than one strain. In each of the remaining 22, more than one strain was examined; some of these latter viruses presented features of special interest, in others the reason for examining more than one strain was mainly to see how far strains presumably identical in character yielded identical features on artificial media; 86 strains belonging to these 22 viruses were examined.

Out of the total 44 new viruses, 2 were classed in Grade I, 3 in Grade II, 1 in Grades II and III, 8 in Grade III, 1 in Grades II and IV, 1 in Grades III and IV, 16 in Grade IV, 7 in Grades IV and V, and 5 in Grade V.

In relation to the original human material the distribution is as follows:—

Grade I.—H 77. R.L. (Liver and Mesenteric Gland); H 89. W.I. (Mesenteric Gland and Lung).

Grade II.—H 100. R.S., H 105. G.S., and H 110. J.B. (all Lupus).

Grades II and III.—H 85. H.B. (Lupus).

Grade III.—H 69. F.K. (Mesenteric Gland and Meninges of Brain); H 72. H.C. (Abscess of Rib); H 88. E.L. (Mesenteric Gland); H 91. H.S., H 101. E.G., H 107. H.H., H 108. H.R., and H 111. S.E. (all Lupus).

Grades II and IV.—H 90. I.P. (Mesenteric Gland and Retroperitoneal Gland).

Grades III and IV.—H 60. W.B. (3 strains from Bronchial Gland in III, 1 strain from Bronchial Gland and strains from Brain, Mesenteric Gland, and Lung in IV).

Grade IV.—H 67. E.P. (Scapular Abscess); H 68. R.B. (Sacral Abscess); H 70. N.W. (Lung, Cervical Gland, Bronchial Glands, and Mesenteric Glands); H 74. D.S. (Mesenteric Gland, Bronchial Gland, and Spleen); H 76. G.M. (Knee Joint); H 82. G.F. (Mesenteric Glands and Lung); H 84. M.S. (Lupus); H 86. C.P. (Meninges of Brain); H 87. W.D. (Suprarenal Capsule); H 93. C.D. (Lung and Acne Spot); H 98. B.R. (Cerebro-spinal Fluid); H. 102. N.H., H 103. N.S., H 106. K.R., H 109. M.W. (all Lupus); H 115. N.S. (Cerebro-spinal Fluid).

Grades IV and V.—H 71. L.V. (Lupus—strain (*a*) in IV, (*b*) in V); H 75. A.D. (Peritoneal Fluid in IV, Lung in V); H 78. O.D. (Bronchial Gland and Lung in IV, Meninges of Brain in V); H 79. J.N. (4 strains from Lung in IV, 8 strains from Lung in V); H 80. D.M. (6 strains from Lung in IV, 3 strains from Lung in V); H 81. P.W. (2 strains from Lung in IV, 7 strains from Lung in V); H 99. L.K. (Lupus—strain from one G.P. in IV, from another in V).

Grade V.—H 83. G.C. (Mesenteric Gland, Bronchial Gland, Meninges of Brain); H 92. D.N. (Lupus); H 104. E.R. (Lung); H 112. B.B., H 114. A.U. (both Lupus).

THE CULTURAL CHARACTERS OF BACILLI ISOLATED FROM SPUTUM.

In addition to the preceding strains Dr. A. S. Griffith has sent to me for examination cultures which he isolated from the sputum of 28 patients. Before collecting the sputum special precautions were taken to avoid the risk of food contamination by living tubercle bacilli of animal origin.

These viruses were numbered H 118—H 145. Twenty-three of them grew luxuriantly on my usual test media; and, on looking over my notes, I find that I have classed them in Grade V in all but one instance, where I described the growth as being equivalent to the best of Grade IV. These 23 viruses call for no further comment.

About the remaining five viruses the results of my observations are as follows:—

H 118. F.C.—Typical of the poorer growths in Grade III.

H 122. F.P.—The growths on potato (3 trials) were no better than the poorer growths of Grade III, but the growths on broth and glycerin-agar were very luxuriant and typical of Grade V.

H 127. R.R.—Typical of Grade I.

H 128. D.D.—Typical of Grade I.

H 133. P.H.—The growths on glycerin-agar (3 trials) were no better than the poorer growths of Grade III, but the growths on broth and potato were equivalent to those of Grade IV.

III.—THE CULTURAL CHARACTERS OF SWINE VIRUSES.

CULTURAL CHARACTERS OF INDIVIDUAL STRAINS.*

VIRUS—P I.

Material Received.—Fifth generation, 8 days old, of culture isolated from original material.

Cultural Characters.—On pure serum good growths were obtained after the first culture, which developed slowly and was rather poor. Broth cultures covered the surface in about five weeks with a thin, semi-transparent pellicle. On glycerin-agar a few colonies became prominent during the second month, but the rest of the surface showed a very thin layer, amounting to little more than a dull grey haze. On potato, after a slight, non-pigmented increase during the first fortnight, the culture ceased to grow.

On glycerin-agar, in a 27 days' film, the bacilli were rather longer than is usual with strains which grow so poorly. They varied in length from 1 to 4 μ; many of them were curved, and they were frequently beaded.

CLASSIFICATION.

Amongst the average growths of Grade I.

VIRUS—P II.

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Twenty-first generation, 5 days old, of culture isolated from original material.

Cultural Characters.—On pure serum a moderate amount of growth was obtained; it was white and distinctly slimy in appearance. On glycerinated serum the growth was more abundant; the surface was well covered in a week with thick, slimy streaks and patches. In broth flasks, containing a layer of glycerinated serum at one side, the bacilli refused to float on the surface of the broth; they grew downwards and formed an abundant deposit at the bottom of the flasks. On glycerin-agar there was an abundant, white, putty-like growth which developed rapidly during the first fortnight and showed no more increase after further incubation. On potato numerous grey colonies appeared during the first week; the bacilli continued to multiply slowly, and at the end of two months the growth was fairly abundant; during the development of the culture yellow pigment was formed.

The bacilli stained like ordinary tubercle bacilli. On horse serum, in a 14 days' film, they were notably short; they varied in length from ·5 to 1·5 μ; forms as long as 1·5 μ were not frequent, and the average was not over ·75 μ; the bacilli were straight and uniformly stained. On glycerinated horse serum the bacilli resembled those grown on pure serum. On glycerin-agar, in a 42 days' film, the majority of the bacilli varied in length from 1 to 3 μ and were straight and uniformly stained; there were a few forms as long as 6 or 7 μ which were curved and stained uniformly.

CLASSIFICATION

A tubercle bacillus of the avian type.

(2.)

Strain.—MONKEY 118. (*Local Lesion.*)

Material Received.—Primary culture, 45 days old, isolated from the local lesion of Monkey 118, which had been inoculated with culture of (1).

Cultural Characters.—On pure serum rather poor growths were obtained, whilst on glycerinated serum the yield was distinctly good; on both media the growth was pale and slimy. On broth the bacilli refused to float. On glycerin-agar an abundant, white, slimy growth was obtained at the end of a week; after the end of the second week there was no further increase. On potato a slow increase was observed until nearly the end of the second month; the yield was fairly abundant and at first cream coloured but afterwards deep yellow.

On horse serum the bacilli resembled, morphologically, those of strain (1) grown on this medium. On bovine serum, in a 15 days' film, in addition to the usual short and straight forms, there were some much longer (3 to 4 μ); these were uniformly stained and notably thick. On glycerin-agar, in a 42 days' film, whilst the majority of bacilli were short, and uniformly stained, much thicker and longer forms (sometimes as long as 6 to 7 μ) were frequent; some of these showed thickened, somewhat club-shaped extremities. On potato, in a 56 days' film, the great majority of the bacilli were from ·75 to 3 μ in length, straight, and uniformly stained.

CLASSIFICATION.

Identical with the preceding strain.

(3.)

Strain.—MONKEY 118. (*Spleen.*)

Material Received.—Primary culture, 45 days old, isolated from the spleen of Monkey 118, which had been inoculated with culture of (1).

Cultural Characters.—Good cultures were obtained on pure serum and on glycerinated serum; on both media the cultures had a dry appearance, which was in contrast to the slimy growth exhibited by the two preceding strains. On broth, in the earlier cultures, some delicate patches were found floating during the second week, but shortly afterwards they all sank; later cultures rapidly covered the surface with a grey speckled pellicle; some of this material sank, but further growth then took place and the flasks remained well covered at the end of two months. On glycerin-agar a thin, grey, semi-opaque layer with many small granular points was slowly formed; little increase took place after the third week, and at the end of two months the total yield was scanty; in contrast to the two preceding strains the growth was, throughout its development, dry. On potato there was hardly any perceptible increase before the end of the third week; at the end of two months there was a scanty grey layer.

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* Most of the swine viruses were derived from submaxillary lymphatic glands; in cases where some other tissue was used as the original material, mention is made of it in my note on "*Material Received.*"

On glycerin-agar, in a 42 days' film, the bacilli were on the whole short and straight (·75 to 3 μ) and many of them were notably thick; on potato they were longer (1 to 5 μ) and frequently curved and beaded.

CLASSIFICATION.

A marked divergence from the avian type exhibited by the two preceding strains, and a general correspondence to the mammalian type with a cultural luxuriance equivalent to the poorer strains in Grade III.

VIRUS—P III.

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Sixteenth generation, 67 days old, of culture isolated from the original material.

Cultural Characters.—A fair amount of growth was obtained on pure serum; on glycerinated serum the yield was much better; on both media it was distinctly slimy. The bacilli refused to grow on the surface of broth. On glycerin-agar a white, slimy, fairly abundant layer was rapidly formed; no increase took place after the second week. On potato grey colonies appeared during the first week; there was afterwards a gradual increase, until near the end of the second month, and a yellow pigment was formed.

The bacilli on the whole were short and straight; some longer and much thicker forms were noted on glycerin-agar.

CLASSIFICATION.

A tubercle bacillus of the avian type.

(2.)

Strain.—G.P. 1612 from CALF 282.

Material Received.—Primary culture, 67 days old, isolated from G.P. 1612, which had been inoculated from Calf 282. The latter animal had been inoculated with culture of (1).

Cultural Characters.—Good growths were obtained on pure serum and on glycerinated serum, and on both media the cultures were dry in appearance. On broth, small islands, some of them fairly thick, covered about a third of the surface in a fortnight; during the following fortnight the surface growth was increased to about twice the amount; it then began to sink, but opaque, finely granular islands remained floating. On glycerin-agar the growth was dry; it grew slowly for about five weeks and became moderately dense and markedly granular; some of the larger granular colonies exhibited a yellow pigment, which faded during the second month. On potato there was a slow, steady increase; during the second month the growth became fairly abundant; about the third week a yellow pigment was observed which afterwards became rather paler.

On potato, in a 56 days' film, the bacilli varied in length from 1 to 6 μ, and were often curved and beaded. On other media the bacilli were shorter (1 to 3 μ) and more generally straight and uniformly stained.

CLASSIFICATION.

A marked divergence from the avian type exhibited by the preceding strain; the general characters are those of mammalian bacilli classed amongst the best growths of Grade III.

VIRUS—P IV.

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Fifth generation, 12 days old, of culture isolated from original material.

Cultural Characters.—On pure serum abundant growths were readily obtained. On broth the surface became covered in five weeks with a thick, somewhat wrinkled and warty pellicle which showed no tendency to sink. On glycerin-agar the growth was rapid, rather moist, thick, and prominently wrinkled. On potato growth commenced vigorously, with the formation of a yellowish brown, crumb-like layer in three weeks; after this period very little further increase took place and the surface never became more than half covered.

On bovine serum, in a 16 days' film, the majority of the bacilli were of the usual type, but a few were longer (3 to 4 μ), curved, and uniformly stained. On glycerin-agar the bacilli, in a 27 days' film, were shorter (1 to 2·5 μ) than is usual for good cultures; but on broth and potato they were of the more usual type, viz., long (1 to 4·5 μ) and curved.

CLASSIFICATION.

Amongst the less abundant growths of Grade V.

(2.)

Strain.—CALF 312.

Material Received.—Fourth generation, 17 days old, of culture isolated from the lung of Calf 312.

Cultural Characters.—Good growths were obtained on pure serum. On broth the surface was covered in three weeks with a thick, warty pellicle which showed no tendency to sink. On glycerin-agar the growth was rapid and became markedly wrinkled and somewhat warty. On potato a fairly abundant yellow growth was obtained ; the pigment afterwards became deeper.

On glycerin-agar, in a 42 days' film, the bacilli varied in length from 1 to 4·5 μ ; they were frequently curved ; globular swellings were rather numerous, but otherwise the staining was uniform.

CLASSIFICATION.

The same position as the preceding strain.

(3.)

Strain.—RABBIT 556 (*Lung*) from CALF 312.

Material Received.—Primary culture, 77 days old, isolated from the lung of Rabbit 556, which had been inoculated with culture from Calf 312.

Cultural Characters.—Good growths were obtained on serum. On broth a grey, moist pellicle grew rapidly and soon began to sink. On glycerin-agar a greyish, scanty layer speckled with some denser points, was slowly formed. On potato the growth was scanty and non-pigmented ; small grey colonies were slowly formed.

CLASSIFICATION.

Amongst the average growths of Grade II.

(4.)

Strain.—RABBIT 556 (*Sternal Gland*) from CALF 312.

Material Received.—Primary culture, 107 days old, isolated from the sternal gland of Rabbit 556, which had been inoculated with culture from Calf 312.

Cultural Characters.—Growth on serum was satisfactory. On broth growth was slow ; a moderately thick layer was formed which never completely covered the surface but showed no tendency to sink. On glycerin-agar a scanty, greyish layer with a few denser points was quickly formed, but no further growth took place and the ultimate yield was very scanty. On potato a scanty, white layer was slowly formed.

The bacilli grown on media other than serum showed only a slight deviation from the serum type.

CLASSIFICATION.

No appreciable difference from the strain isolated from the lung.

(5.)

Strain.—RABBIT 557 from CALF 312.

Material Received.—Sixth generation, 7 days old, of culture isolated from Rabbit 557, which had been inoculated with culture from Calf 312.

Cultural Characters.—Growths on serum were satisfactory. On broth the surface became covered in three weeks with an opaque, moderately dense layer which shortly afterwards became moist and sank. On glycerin-agar a moderately dense, granular layer was obtained. On potato the growth was not very copious but exhibited a yellow pigment.

The bacilli, on various media, only exhibited a slight divergence from the serum type.

CLASSIFICATION.

Amongst the better growths of Grade III, but not equal to the best.

VIRUS—P V.

Material Received.—Second generation, 26 days old, of culture isolated from original material.

Cultural Characters.—Good growths were obtained without difficulty on pure serum. On broth the surface was covered in a month with a very thin pellicle which during the fifth week became moist and began to sink. A scanty, moist, greyish layer was formed on glycerin-agar during the second and third weeks ; no further change was noted until the eighth week, when some denser grey points and patches appeared. On potato there was a scanty cream coloured growth.

CLASSIFICATION.

Amongst the poorer growths of Grade II.

VIRUS—P VI.

Material Received.—A portion of the spleen of G.P. 1515, which had been inoculated with the original material (inguinal gland). In a smear made from this material bacilli were not very numerous; a few were found in one out of every five or six microscopic fields. They measured from 2 to 3·5 μ in length and were generally curved and beaded.

Cultural Characters.—On bovine serum inoculated with an emulsion of the spleen small groups of very minute translucent colonies were just visible in eight days; at the end of a fortnight there were large areas of minute, nearly translucent colonies closely massed together; the growth was then uniformly distributed by means of the platinum loop and a week later had formed all over the surface a continuous, grey, rather thin layer. The first subculture, made from a 13 days' old primary culture, grew well and rapidly. Three tubes of glycerinated bovine serum were inoculated with an emulsion of the spleen, but not a trace of growth was visible after two months' incubation. On broth the growth was very thin and showed a tendency to become moist and sink. On glycerin-agar a thin grey layer was formed, and on potato there was a scanty white growth.

CLASSIFICATION.

Amongst the poorer growths of Grade II.

VIRUS—P VII.

Material Received.—Second generation, 18 days old, of culture isolated from original material.

Cultural Characters.—The first and second subcultures on serum grew poorly, but later cultures were satisfactory. On broth the growth was slow, and the surface never became completely covered; the pellicle was slightly opaque, and thin on the whole, but with some denser points. On glycerin-agar a very slow increase was maintained during the first six weeks and then ceased; the ultimate yield was a thin, opaque, grey, somewhat frosted layer, amongst which were scattered minute grey elevations. No perceptible growth was obtained on potato.

The bacilli grown on glycerin-agar, in a 44 days' film, exhibited great irregularities of staining and measured from 1 to 5 μ.

CLASSIFICATION.

Amongst the average growths of Grade II.

VIRUS—P VIII.

Material Received.—Primary culture, 27 days old, isolated from original material.

Cultural Characters.—Good growths were obtained on pure serum without difficulty. The broth cultures were covered at the end of a month, and though thin on the whole exhibited some denser patches. On glycerin-agar a slow increase was maintained during the first six weeks, the ultimate yield being a moderately dense, frosted glass type of growth with some denser patches. On potato the growth was pale and scanty.

The bacilli grown on glycerin-agar, in a 15 days' film, were short (1 to 2·5 μ), generally straight, and for the most part uniformly stained.

CLASSIFICATION.

Amongst the poorer growths of Grade III.

VIRUS—P IX.

Material Received.—Primary culture, 54 days old, isolated from original material.

Cultural Characters.—After the first subculture, which was very poor, satisfactory growths were obtained on serum. On broth the surface was covered in about 5 weeks with a pellicle which was in part extremely thin and transparent and in part thick, moist, and with a marked tendency to sink. On glycerin-agar the increase was very slow but continued for 7 weeks, the ultimate yield being a thin, frosted glass type of growth with denser pin points. On potato a scanty, non-pigmented growth was obtained.

On broth, in a 35 days' film, the bacilli varied in length from ·5 to 4·5 μ; coccal forms were frequent; and amongst the longer forms globular swellings, cylindrical thickenings and irregular staining were very frequent. On glycerin-agar the bacilli were rather long (1 to 5 μ) and on potato, in a 36 days' film, the majority were unusually long (4 to 8 μ).

CLASSIFICATION.

Amongst the better growths of Grade II.

VIRUS—P X.

Material Received.—Third generation, 20 days old, of culture isolated from original material (mesenteric gland).

Cultural Characters.—On pure serum good growths were obtained. On broth a thin, opaque pellicle was formed which became moist and sank before the surface was completely covered. On glycerin-agar a thin, greyish, somewhat flaky, moist layer was very slowly formed. On potato the growth was slow scanty, and non-pigmented.

The bacilli grown on media other than serum were from 1 to 3·5 μ in length and were on the whole uniformly stained.

CLASSIFICATION.

Amongst the average growths of Grade II.

VIRUS—P XI.

Material Received.—Primary culture on egg, 22 days old, isolated from original material.

Cultural Characters.—Good growths were obtained on pure serum. On broth large moist patches were formed rapidly but sank before they had succeeded in covering the surface. On glycerin-agar a thin, moist, grey, irregular layer, showing some denser points and patches, was slowly formed. On potato the surface was not uniformly covered but several fairly large, slightly yellow colonies were formed.

The bacilli grown on potato, in a 53 days' film, varied in length from 1 to 6 μ, all intermediate lengths being found. Except the shortest forms, the bacilli were curved and beaded.

CLASSIFICATION.

Amongst the better growths of Grade II.

VIRUS—P XII.

Material Received.—Third generation, 14 days old, of culture isolated from G.P. 1636, which had been inoculated with the original material.

Cultural Characters.—The growth on pure serum was satisfactory but not particularly abundant. On broth a grey, rather thick, moist pellicle was formed but sank before the surface was more than half covered. On glycerin-agar a thin grey layer, rather moist and with some denser patches, was slowly formed. On potato the growth was very scanty and non-pigmented.

The bacilli grown on potato, in a 60 days' film, were very irregular both in length (·5 to 5 μ) and in staining properties.

CLASSIFICATION.

Amongst the average growths of Grade II.

VIRUS—P XIII.

Material Received.—Primary culture, 41 days old, isolated from original material.

Cultural Characters.—Good growths were obtained from the commencement on pure serum. On broth the growth was slow and consisted of rather dense grey islands which became moist and sank before the whole of the surface was covered. On glycerin-agar growth was slow and scanty, yielding a very thin, grey, uniform, moist layer. On potato there was a scanty, non-pigmented growth.

The bacilli grown on potato, in a 36 days' film, varied in length from 3 to 6 μ and were nearly all curved and either regularly beaded or irregularly stained.

CLASSIFICATION.

Amongst the poorer growths of Grade II.

VIRUS—P XIV.

Material Received.—A sternal gland of G.P. 1624 which had been inoculated with the original material.

Cultural Characters.—Cultures were raised on egg and on pure serum (dog), numerous minute colonies becoming visible at the end of three weeks. No growth was obtained on glycerinated serum inoculated with the same material. Subcultures grew satisfactorily on pure serum. On broth a thin, slightly opaque layer had covered the surface in three weeks, and then commenced growing up the glass; a fortnight afterwards the pellicle became moist and sank. On glycerin-agar the surface rapidly became covered with a thin, greyish growth, forming a homogeneous, slightly opaque film; with the exception of a few dense white points which subsequently appeared, very little further increase took place. On potato a scanty, white growth appeared during the second week; slight increase took place during the second month, and the growth assumed a crumb-like appearance.

The bacilli grown on media other than serum were very irregular both in length and staining properties.

CLASSIFICATION.

Amongst the better growths in Grade II.

VIRUS—P XV.

Material Received.—Fifth generation, 14 days old, of culture isolated from original material.

Cultural Characters.—Good growths were obtained on pure serum. On broth the surface was covered with a semi-transparent pellicle in three weeks; denser points and small patches developed subsequently, but at the end of five weeks the growth began to sink. On glycerin-agar a thin, grey layer developed rapidly, but although many small, raised, white colonies appeared subsequently no general thickening took place. On potato there was a scanty, non-pigmented growth.

The bacilli grown on potato, in a 49 days' film, were longer (1 to 5 μ) than on other media, and the majority of them were stained uniformly.

CLASSIFICATION.

Amongst the average growths in Grade II.

VIRUS—P XVI.

Material Received.—Second generation, 9 days old, of culture isolated from original material (bronchial gland).

Cultural Characters.—Good growths were obtained on pure serum. On broth the growth was slow and semi-translucent; the surface became covered during the seventh week. On glycerin-agar the surface readily became covered with a very thin, grey layer, but only slight increase in density took place subsequently. On potato the surface gradually became covered with a uniform, rather scanty, grey layer.

On potato the bacilli became long (1 to 4 μ) and irregularly stained, but on other media they only deviated slightly from the serum type.

CLASSIFICATION.

Amongst the poorer growths in Grade II.

VIRUS—P XVII.

Material Received.—Second generation, 21 days old, of culture isolated from original material (a mesenteric gland).

Cultural Characters.—Good growths were obtained on pure serum. On broth some difficulty was found in starting the growth; in six weeks the surface became covered with a thin, irregular pellicle, showing some small, rather dense, moist patches. On glycerin-agar a grey haze only was obtained. Potato cultures showed no growth visible to the naked eye, but on microscopic examination some evidence of increase was found.

CLASSIFICATION.

Amongst the poorer growths in Grade I.

VIRUS—P XVIII.

Material Received.—Second generation, 9 days old, of culture isolated from original material (lung).

Cultural Characters.—Good growths were obtained on pure serum. On broth growth was slow; in six weeks the surface was covered with an almost transparent film which refused to thicken. On glycerin-agar a thin, greyish layer with a few denser points was formed. On potato a scanty, white layer was obtained.

Only a slight deviation from the serum type was found in bacilli grown on other media.

CLASSIFICATION.

Amongst the rather better growths in Grade I.

VIRUS—P XIX.

Material Received.—Seventh generation, 34 days old, of culture isolated from original material.

Cultural Characters.—On pure serum the growth of this bacillus was poor, both in the earliest and in subsequent generations. On broth several attempts were made, but no more than a few minute floating islands were obtained. On glycerin-agar no more than a faint greyish haze was formed. On potato there was no growth visible to the naked eye, but on microscopic examination distinct evidence of growth was obtained.

CLASSIFICATION.

One of the poorest growths in Grade I.

VIRUS—P XX.

Material Received.—Primary culture, on egg, 41 days old, isolated from original material.

Cultural Characters.—The growths on pure serum were poor, even after several subcultures. On broth a few small islands were obtained, but no continuous growth. On glycerin-agar there was no more than a fine grey haze. On potato there was no definite appearance of growth to the naked eye, but there were definite signs of growth microscopically.

CLASSIFICATION.

One of the poorest growths in Grade I.

VIRUS—P XXI.

Material Received.—Seventeenth generation, 10 days old, of culture isolated from original material.

Cultural Characters.—Growth was very poor on pure serum, but on glycerinated serum, after one or two subcultures, good growths were readily obtained, and were of a typical white, slimy appearance. On broth the bacilli refused to float. On glycerin-agar a white, slimy growth developed, and attained its maximum in about a fortnight. On potato a typical, fairly abundant, yellow, moist growth was slowly formed.

Classcifiation.

Characteristic of the avian bacillus.

VIRUS—P XXII.

Material Received.—Fourth generation, 17 days old, of culture isolated from original material.

Cultural Characters.—Good growths were obtained on pure serum. On broth growth was very slow and consisted of discrete, scattered islands; at the end of two months the surface was about two-thirds covered. On glycerin-agar a very scanty, greyish layer was obtained. On potato a few white colonies were produced.

Classification.

Rather better than the worst growths, but distinctly inferior to the best growths in Grade I.

VIRUS—P XXIII.

Material Received.—Third generation, 17 days old, of culture isolated from original material (bronchial gland).

Cultural Characters.—Good growths were obtained on pure serum. On broth growth was very poor, no more than small, discrete islands being obtained. On glycerin-agar there was a very scanty, greyish layer. On potato a scanty non-pigmented growth was visible.

The bacilli grown on potato, in a 60 days' film, showed a marked divergence from the serum type, being longer (·75 to 4·5 μ) and generally beaded or irregularly stained.

Classification.

Amongst the poorer growths in Grade I.

VIRUS—P XXIV.

Material Received.—Second generation, 10 days old, of culture isolated from G.P. 1790, which had been inoculated with original material.

Cultural Characters.—Fairly good growths were obtained on pure serum. On broth the surface was covered in about three weeks with a grey, rather thin pellicle which shortly afterwards become moist and sank. On glycerin-agar a scanty, greyish layer, with some denser streaks, was slowly formed. On potato there was a scanty layer with some greyish yellow colonies.

The bacilli grown on potato were distinctly longer than the serum type.

Classification.

Amongst the poorer growths of Grade II.

VIRUS—P XXV.

Material Received. — Primary culture, on egg, 34 days old, isolated from original material.

Cultural Characters. — Growths on pure serum, even after subculture for some generations, were poor. On broth several attempts were made, but all failed to produce more than a few minute islands. On glycerin-agar no more than a faint grey haze was obtained. On potato very little sign of growth was perceptible to the naked eye, but on microscopic examination it was clear that the bacilli had multiplied and become longer than serum bacilli.

Classification.

One of the poorest growths in Grade I.

VIRUS—P XXVI.

Material Received. — Primary culture, on egg, 30 days old, isolated from original material.

Cultural Characters. — Growths on pure serum were poor, even after subculture for several generations. On broth the surface was half covered in three weeks with a thin semi-opaque pellicle which shortly afterwards became moist and sank. On glycerin-agar no more than a grey haze was obtained. On potato there was a slight amount of growth, and the bacilli were rather longer than those grown on serum.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

VIRUS—P XXVII.

Material Received. — Primary culture, on egg, 34 days old, isolated from original material.

Cultural Characters.—On pure serum the growth was very poor. On glycerinated serum a raised, slimy growth appeared in the first week; the culture was kept up on this medium. On broth, with the exception of a few small, moist islands which appeared during the second month, no surface growth was obtained. On glycerin-agar the growth was abundant, white, and slimy, and obtained its maximum in about a fortnight. On potato a fairly abundant layer was obtained; increase was slow, and the colour changed from grey to yellow during the second month.

On glycerin-agar, in a 42 days' film, the majority of the bacilli were very little longer than serum grown bacilli but stained more irregularly; there were also present a considerable number of much longer (4 to 6 μ) and thicker forms; some of these last exhibited irregular, oval, or club-shaped thickenings in some parts of their length. On potato, in a 56 days' film, the bacilli varied in length from 1 to 5 or 6 μ, and were generally curved and beaded; exceptionally thick forms were not noted.

CLASSIFICATION.

Characteristic of the avian tubercle bacillus.

VIRUS—P XXIX.

Material Received.—Second generation, 10 days old, of culture isolated from original material.

Cultural Characters.—Good growths were obtained on serum. On broth growth was scanty, and was obtained with difficulty; it consisted of small, discrete, semi-translucent islands. On glycerin-agar very little more than a grey haze was obtained. On potato a very scanty, white layer was formed.

The bacilli grown on potato varied in length from 1 to 4 μ and were almost all straight and uniformly stained.

CLASSIFICATION.

Amongst the poorer but not amongst the worst growths in Grade I.

VIRUS—P XXX.

Material Received.—Second generation, 10 days old, isolated from original material.

Cultural Characters.—On serum growth was rather poor at first but improved after subculture. On broth growth was slow; scattered islands were formed which gradually thickened but failed to spread as a continuous pellicle; the total yield was scanty. On glycerin-agar a little more than a grey haze was formed. On potato growth was very scanty.

The bacilli grown on media other than serum closely resembled the serum type.

CLASSIFICATION.

One of the better growths in Grade I.

VIRUS—P XXXI.

Material Received.—Second generation, 10 days old, isolated from original material.

Cultural Characters.—Growth on serum was rather poor at first, but became good after subculture. On broth the surface was covered in rather less than a month with a thin, semi-transparent pellicle, which subsequently became moist and sank. On glycerin-agar growth was scanty; at the end of two months there were a few grey streaks and patches and one large, raised, brownish-grey colony, but over the rest of the surface there was nothing more than a grey haze. On potato a scanty, white growth was slowly produced.

Except that those grown on broth stained very irregularly, the bacilli grown on the various media exhibited only a slight deviation from the serum type.

CLASSIFICATION.

Amongst the best growths of Grade I

VIRUS—P XXXII.

Material Received.—Second generation, 14 days old, of culture isolated from original material.

Cultural Characters.—Growth on serum was satisfactory. On broth the surface was about two-thirds covered in a month with an opaque, fairly thick pellicle which was evidently of the moist type; shortly afterwards it sank. On glycerin-agar growth commenced as a grey haze sprinkled with numerous denser points. These denser points gradually developed into raised colonies resembling the casts of earth-worms. On potato a scanty grey layer was slowly formed.

CLASSIFICATION.

Amongst the poorest growths in Grade III.

VIRUS—P XXXIII.

Material Received.—Primary culture, 35 days old, isolated from the original material.

Cultural Characters.—Growth on serum was satisfactory. On broth the surface became covered in about three weeks with an opaque uniform pellicle; the pellicle then gradually thickened, and exhibited patches of denser growth; it remained floating for about six weeks, but afterwards showed a tendency to sink. On glycerin-agar numerous raised discrete colonies were slowly formed; the rest of the surface was poorly covered. On potato the surface became covered with a layer which was scanty on the whole, but exhibited many small, grey, granular colonies.

On potato the bacilli varied in length from 1 to 4 μ, and were generally straight and uniformly stained. On other media the bacilli exhibited only a slight deviation from the serum type.

CLASSIFICATION.

About the middle of Grade III.

VIRUS—P XXXIV.

Material Received.—Second generation, 10 days old, of culture isolated from original material.

Cultural Characters.—Growth on serum was satisfactory. On broth growth was very poor; on one culture a small island was formed which made little surface extension, but became very thick and showed a tendency to grow downwards; in another flask a few small islands were formed in five weeks; they showed a tendency to thicken rather than to spread over the surface. On glycerin-agar no more than a grey haze was formed. On potato there was a slight growth which could be recognised microscopically, but was hardly visible to the naked eye.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

VIRUS—P XXXVI.

Material Received.—Eighth generation, 23 days old, of culture isolated from original material.

Cultural Characters.—Growth on serum was satisfactory. On broth a grey pellicle with some thicker patches commenced to form rapidly, and had covered two-thirds of the surface in three weeks; it then became moist and sank. On glycerin-agar no more than a thin, slightly opaque layer was formed. On potato the growth was scanty, but exhibited a few yellow colonies at the end of two months.

The bacilli, on various media, exhibited only a slight divergence from the serum type.

CLASSIFICATION.

Amongst the poorer growths of Grade II.

VIRUS—P XXXVII.

Material Received.—Seventh generation, 23 days old, of culture isolated from original material.

Cultural Characters.—Growth on serum was satisfactory. On broth the surface was covered in three weeks with a grey pellicle containing denser patches; by the sixth week these denser patches had all fallen to the bottom, but the thinner material remained floating. On glycerin-agar the surface became fairly well covered with a grey layer presenting a granular, frosted appearance. On potato the surface became covered with a grey, slightly granular layer.

The bacilli, on media other than serum, varied in length from 1 to 3·5 μ; curved or irregularly stained forms were not very numerous.

CLASSIFICATION.

Amongst the better growths of Grade III.

VIRUS—P XXXVIII.

Material Received.—Second generation, 24 days old, of culture isolated from original material.

Cultural Characters.—Good growths were obtained on serum. On broth growth was slow; a mottled, moderately dense pellicle covered two-thirds of the surface in six weeks; it was of the moist type and readily sank. On glycerin-agar a semi-opaque layer, with some denser, slightly granular patches, was formed during the first fortnight, but exhibited no subsequent increase. On potato a very scanty non-pigmented layer was slowly produced.

On horse serum, in a 15 days' film, the bacilli stained irregularly, and some curved and beaded forms, 3 to 4 μ in length, were noted. On broth, in a 24 days' film, the bacilli varied in length from ·75 to 2 μ, and were for the most part straight and uniformly stained. On glycerin-agar, in a 42 days' film, the bacilli were slightly longer and less regularly stained than on broth. On potato, in a 56 days' film, the majority of the bacilli were short, straight, and uniformly stained; but longer (up to 5 μ), curved, and beaded forms were also present.

CLASSIFICATION.

About the middle of Grade II.

VIRUS—P XXXIX.

Material Received.—Second generation, 24 days old, of culture isolated from original material.

Cultural Characters.—Fairly good growths were obtained on serum. On broth the growth was delicate and semi-opaque; it sank before the surface was more than half covered. On glycerin-agar a fairly dense, granular layer was slowly formed. On potato the surface was covered with a grey layer exhibiting numerous small, raised colonies.

The bacilli grown on potato, in a 56 days' film, varied in length from 1 to 6 μ and were mostly curved and beaded; those grown on other media only diverged slightly from the serum type.

CLASSIFICATION.

Intermediate between the poorer and the better growths of Grade III.

VIRUS—P XL.

Material Received.—Second generation, 24 days old, of culture isolated from original material.

Cultural Characters.—The growths on serum were rather poor. On broth the surface became covered in less than three weeks with a thin, greyish pellicle which soon afterwards became moist and sank. On glycerin-agar a scanty, greyish layer with a few denser patches was formed. On potato the growth was very scanty and non-pigmented.

The bacilli, on various media, exhibited only a slight divergence from the serum type.

CLASSIFICATION.

Amongst the average growths of Grade II.

VIRUS—P XLI.

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Second generation, 24 days old, of culture isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth a thick pellicle was slowly formed and covered the surface in about five weeks; the pellicle gradually increased in density and, at the end of two months, showed no tendency to sink. On glycerin-agar a uniform, fairly dense, very granular, and slightly wrinkled layer was formed during the first month; no further change took place. On potato the growth was moderately abundant and exhibited a brown pigment.

On media other than serum the bacilli varied in length from ·75 to 3·5 μ and were generally stained uniformly.

CLASSIFICATION.

Amongst the poorest growths of Grade IV.

(2.)

Strain.—PIG 166. (*Lung.*)

Material Received.—Third generation, 14 days old, of culture isolated from Pig 166.

Cultural Characters.—Good growths were obtained on serum. On broth growth was slow during the first fortnight, but at the end of a month the surface was well covered; during the second month the pellicle became very thick and showed no tendency to sink On glycerin-agar the growth was fairly dense, finely granular and somewhat wrinkled. On potato there was a moderate amount of growth, showing a bright yellow pigment.

CLASSIFICATION.

Equal, on the whole, to an average growth of Grade IV.

(3.)

Strain.—PIG 202.

Material Received.—Primary culture, 60 days old, isolated from Pig. 202.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered with a thick, uniform layer in a month. On glycerin-agar the growth was abundant and became markedly wrinkled but not warty. On potato a thick, brownish-red, raised growth was obtained.

The bacilli grown on media other than serum were notably long.

CLASSIFICATION.

About the average of Grade V.

VIRUS—P XLII.

Material Received.—Third generation, 17 days old, of culture isolated from the original material.

Cultural Characters.—The growths on serum were fairly good. On broth the surface became covered in a fortnight with a grey, semi-opaque layer which afterwards thickened in patches and then became moist and sank. On glycerin-agar increase was slow but steadily maintained, and the ultimate yield was fairly thick and of the granular type. On potato the surface became gradually covered with a moderately thick, non-pigmented layer, containing many small, raised colonies.

The bacilli on broth and glycerin-agar diverged slightly, and those on potato markedly, from the serum type.

CLASSIFICATION.

Amongst the better growths of Grade III. but not equal to the best.

VIRUS—P XLIII.

Material Received.—Second generation, 24 days old, of culture isolated from the original material.

Cultural Characters.—The growths on serum were fairly satisfactory, but never particularly good. On broth the first two cultures failed to grow; a third culture slowly produced a thin, speckled pellicle covering rather more than half the surface. On glycerin-agar a grey haze with a few slightly denser patches was formed. On potato there was a scanty layer covering most of the surface.

The bacilli grown on horse serum, in a 15 days' film, stained less regularly than usual; many beaded forms were noted.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

VIRUS—P XLIV.

Material Received.—Second generation, 17 days old, of culture isolated from original material.

Cultural Characters.—On pure serum growth was poor; on glycerinated serum it was good; on both media the growth had a slimy appearance. The culture was kept up on glycerinated serum. On broth no surface growth was obtained. On glycerin-agar a white, slimy growth developed and attained its maximum in about a fortnight. On potato a fairly abundant, raised, white growth was formed; no development of pigment was noted.

On glycerin-agar, in a 42 days' film, the majority of the bacilli were very little longer than serum grown bacilli, but their staining was more irregular; much longer (4 to 6 μ) and thicker bacilli were also present in considerable numbers. On potato, in a 56 days' film, the bacilli varied in length from 1 to 3·5 μ; they were generally straight and often beaded.

CLASSIFICATION.

Characteristic of the avian tubercle bacillus.

VIRUS—P XLVI.

Material Received.—Second generation, 33 days old, of culture isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth an opaque, rather thin pellicle covered most of the surface in three weeks; the pellicle then gradually became denser; it showed no sign of sinking at the end of two months. On glycerin-agar a grey layer containing unequally distributed thickened patches was formed; these patches became granular but not wrinkled. On potato a fairly abundant yellowish growth was obtained.

CLASSIFICATION.

Amongst the best growths of Grade III.

VIRUS—P XLVII.

Material Received.—Second generation, 14 days old, of culture isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered in less than a month with an opaque layer containing denser patches; some of the pellicle showed a tendency to sink, but growth was then renewed, and at the end of two months the surface was covered with a grey layer of unequal density which showed no further tendency to sink. On glycerin-agar growth was unequal; some rather dense colonies and patches were developed, but the rest of the surface was poorly covered. On potato there was a thin layer which spread over all the surface and exhibited many minute colonies.

The bacilli, on various media, exhibited only a slight divergence from the serum type.

CLASSIFICATION.

Intermediate between the poorer and the better growths of Grade III.

VIRUS—P XLIX.

Material Received.—Fifth generation, 13 days old, of culture isolated from the original material.

Cultural Characters.—Growth on serum was satisfactory. On broth fairly large dense islands were formed in one culture, but these soon became moist and sank; in another culture a moderately thick and granular pellicle was slowly formed, and showed no tendency to sink. On glycerin-agar the surface readily became covered with a fairly dense granular growth. On potato there was a moderate amount of growth, which was raised and cream coloured.

The bacilli, on various media, only exhibited a slight divergence from the serum type.

CLASSIFICATION.

Amongst the better growths of Grade III.

VIRUS—P L.

Material Received.—Fifth generation, 13 days old, of culture isolated from the original material.

Cultural Characters.—Growth on serum was satisfactory. On broth growth was obtained with some difficulty; islands covering about a third of the surface were rapidly formed and were rather dense, but they soon became moist and sank, no further surface growth being obtained. On glycerin-agar a scanty greyish layer, rather more than a grey haze, was obtained. On potato growth was scanty, but there were a few slightly raised, greyish-yellow colonies.

CLASSIFICATION.

One of the better growths in Grade I.

VIRUS—P LI.

Material Received.—Seventh generation, 13 days old, of culture isolated from the original material.

Cultural Characters.—Growths on serum were satisfactory. On broth the surface was covered in a month with a delicate, transparent pellicle mottled with thick, moist, circular patches; all the pellicle had fallen to the bottom at the end of the sixth week. On glycerin-agar, in one culture, a few warty colonies were present at the end of two months, the rest of the surface being poorly covered; in another culture a scanty greyish layer, with some denser streaks, was obtained. On potato a thin layer of growth was formed.

The bacilli grown on media other than serum exhibited only a slight deviation from the serum type.

CLASSIFICATION.

Amongst the growths of Grade II, but not equal to the best.

VIRUS—P LII.

Material Received.—Fifth generation, 13 days old, of culture isolated from the original material.

Cultural Characters.—Growths on serum were satisfactory. On broth growth was moderately rapid, of unequal density, and showed a tendency to become moist and sink. On glycerin-agar the growth was irregularly distributed, being thick and granular in some patches but poor elsewhere; in another culture it showed a tendency to form numerous isolated warty colonies, with very little growth between them. On potato there were some raised yellowish colonies, but over most of the surface the growth was scanty and non-pigmented.

CLASSIFICATION.

Amongst the intermediate growths of Grade III.

VIRUS—P LIII.

Material Received.—Seventh generation, 14 days old, of culture isolated from the original material.

Cultural Characters.—Growths on serum were fairly good. On broth no growth was obtained in two flasks; in a third several small, thin islands were formed slowly, and the surface became nearly covered with scanty, delicate material in two months. On glycerin-agar there was no more than a grey haze. On potato growth was very scanty.

The bacilli grown on glycerin-agar were unusually long (1 to 5 μ and occasionally longer) and were often curved and beaded.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

VIRUS—P LIV.

Material Received.—Sixth generation, 14 days old, of culture isolated from the original material.

Cultural Characters.—Fairly good growths were obtained on serum. On broth a few thin, scattered islands were obtained with difficulty and gradually spread over most of the surface. On glycerin-agar very little more than a grey haze was formed. On potato growth was very scanty and non-pigmented.

The bacilli grown on glycerin-agar resembled those of P LIII.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

VIRUS—P LV.

Material Received.—Sixth generation, 14 days old, of culture isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth thin islands, speckled with white colonies, slowly formed but failed to completely cover the surface. On glycerin-agar there was a very scanty layer, amounting to little more than a grey haze. On potato there was a definite though scanty layer.

As with the two preceding viruses, the bacilli grown on glycerin-agar were notably long.

CLASSIFICATION.

Grade I.; perhaps slightly better than P LIII and P LIV.

VIRUS—P LVI.

Material Received.—Primary culture, 47 days old, isolated from the original material (a subcutaneous nodule).

Cultural Characters.—The growth on serum was fairly good. On broth no more than a few scattered islands were obtained. On glycerin-agar no more than a grey haze was formed. On potato the growth was very scanty.

The bacilli grown on potato, in a 56 days' film, varied in length from 1 to 6 μ and were generally curved and uniformly stained.

CLASSIFICATION.

One of the poorest growths in Grade I.

VIRUS—P LVII.

Material Received.—Sixth generation, 20 days old, of culture isolated from original material (a bronchial gland).

Cultural Characters.—Good growths were obtained on serum. On broth three cultures were tried but yielded no more than a few thin, scattered islands of various sizes which covered about half the surface at the end of two months. On glycerin-agar there was no more than a grey haze, with a few slightly denser streaks. On potato there was a very scanty, non-pigmented growth.

The bacilli on media other than serum showed only a slight divergence from the serum type.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

VIRUS—P LVIII.

Material Received.—Sixth generation, 20 days old, of culture isolated from original material (a mammary gland).

Cultural Characters.—Good growths were obtained on serum. On broth the surface became rather more than half covered towards the end of the fourth week, but the pellicle then became moist and sank. On glycerin-agar growth at the end of the first fortnight consisted of a thin grey layer; this failed to thicken, but during the second month many discrete, granular colonies appeared; between these colonies there was very little growth. On potato a thinnish layer was slowly formed, without any definite pigmentation.

On broth and glycerin-agar the bacilli were much longer (1 to 4 μ or more) than on serum, and on glycerin-agar their shape was very irregular.

CLASSIFICATION.

Amongst the best growths of Grade II.

VIRUS—P LIX.

Material Received.—Sixth generation, 20 days old, of culture isolated from original material (synovial membrane from a joint).

Cultural Characters.—Good growths were obtained on serum. On broth three cultures were tried, but no more than a few thin, scattered islands were obtained; these covered about half the surface at the end of two months. On glycerin-agar there was a grey haze with a few slightly denser streaks. On potato growth was very scanty and non-pigmented.

On potato a few of the bacilli were from 4 to 5 μ in length, straight and beaded; but the majority resembled serum grown bacilli.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

VIRUS—P LX.

Material Received.—Fifth generation, 20 days old, of culture isolated from original material (a spleen).

Cultural Characters.—Good growths were obtained on serum. On broth three cultures were tried, but no more than a few thin, scattered islands were obtained, the surface not being more than half covered at the end of two months. On glycerin-agar there was little more than a grey haze. On potato growth was very scanty and non-pigmented.

The bacilli grown on media other than serum showed only a slight divergence from the serum type.

CLASSIFICATION.

Amongst the poorer growths of Grade I.

VIRUS—P LXI.

Material Received.—Fourth generation, 20 days old, of culture isolated from original material (a bronchial gland).

Cultural Characters.—Good growths were obtained on serum. On broth large, thin, grey patches were formed during the third week, but after the fifth week these became moist and sank. On glycerin-agar a thin grey layer was formed during the first fortnight; slight patches of thickening were afterwards produced, but the total yield was poor. On potato there was a rather thin layer; a little yellow pigmentation was seen in the earlier stages of the growth, but the colour afterwards disappeared.

On media other than serum the bacilli showed a marked divergence from the serum type. Length, 1 to 5 μ or longer; beading and irregular staining frequent.

CLASSIFICATION.

Amongst the average growths of Grade II.

VIRUS—P LXII.

Material Received.—Fourth generation, 20 days old, of culture isolated from original material (spleen).

Cultural Characters.—Good growths were obtained on serum. On broth the surface became covered in a month with a thin grey layer which showed a marked tendency to sink. On glycerin-agar a thin grey layer was slowly formed, and out of this some denser colonies and patches developed during the second month. On potato the surface became covered with a scanty but definite layer, which showed a slight yellow pigmentation at first, but this afterwards disappeared.

On media other than serum the bacilli were notably long (1·5 to 5 μ, and occasionally 6 to 8 μ) and were frequently beaded.

CLASSIFICATION.

Amongst the best growths of Grade II.

CLASSIFICATION OF SWINE STRAINS.

(1.) MAMMALIAN.

Grade.	Cultural Vigour Relative to Grade.	Strain.	Virus.	Original Porcine Material.
I.	Below average	Original Material	P XVII.	Mesenteric gland.
„	„	Original Material	P XIX.	Submaxillary lymphatic gland.
„	„	Original Material	P XX.	Submaxillary lymphatic gland.
„	„	Original Material	P XXIII.	Bronchial gland.
„	„	Original Material	P XXV.	Submaxillary lymphatic gland.
„	„	Original Material	P XXVI.	Submaxillary lymphatic gland.
„	„	Original Material	P XXXIV.	Submaxillary lymphatic gland.
„	„	Original Material	P XLIII.	Submaxillary lymphatic gland.
„	„	Original Material	P LIII.	Submaxillary lymphatic gland.
„	„	Original Material	P LIV.	Submaxillary lymphatic gland.
„	„	Original Material	P LVI.	Subcutaneous nodule.
„	„	Original Material	P LVII.	Bronchial gland.
„	„	Original Material	P LIX.	Synovial membrane.
„	„	Original Material	P LX.	Spleen.
„	Average	Original Material	P I.	Submaxillary lymphatic gland.
„	„	Original Material	P XXII.	Submaxillary lymphatic gland.
„	„	Original Material	P XXIX.	Submaxillary lymphatic gland.
„	„	Original Material	P LV.	Submaxillary lymphatic gland.
„	Above average	Original Material	P XVIII.	Lung.
„	„	Original Material	P XXX.	Submaxillary lymphatic gland.
„	„	Original Material	P XXXI.	Submaxillary lymphatic gland.
„	„	Original Material	P L.	Submaxillary lymphatic gland.
II.	Below average	Original Material	P V.	Submaxillary lymphatic gland.
„	„	G. P. 1515 from Original Material	P VI.	Inguinal gland.
„	„	Original Material	P XIII.	Submaxillary lymphatic gland.
„	„	Original Material	P XVI.	Bronchial gland.
„	„	G. P. 1790 from Original Material	P XXIV.	Submaxillary lymphatic gland.
„	„	Original Material	P XXXVI.	Submaxillary lymphatic gland.
„	Average	Rabbit 556 (Lung) from Calf 312	P IV.	Submaxillary lymphatic gland.
„	„	„ (Sternal Gland) „	„	„ „ „
„	„	Original Material	P VII.	Submaxillary lymphatic gland.
„	„	Original Material	P X.	Submaxillary lymphatic gland.
„	„	G. P. 1636 from Original Material	P XII.	Submaxillary lymphatic gland.
„	„	Original Material	P XV.	Submaxillary lymphatic gland.
„	„	Original Material	P XXXVIII.	Submaxillary lymphatic gland.
„	„	Original Material	P XL.	Submaxillary lymphatic gland.
„	„	Original Material	P LI.	Submaxillary lymphatic gland.
„	„	Original Material	P LXI.	Bronchial gland.
„	Above average	Original Material	P IX.	Submaxillary lymphatic gland.
„	„	Original Material	P XI.	Submaxillary lymphatic gland.
„	„	G. P. 1624 from Original Material	P XIV.	Submaxillary lymphatic gland.
„	„	Original Material	P LVIII.	Mammary gland.
„	„	Original Material	P LXII.	Spleen.
III.	Below average	Monkey 118 (Spleen)	P II.	Submaxillary lymphatic gland.

CLASSIFICATION OF SWINE STRAINS—*continued.*

Grade.	Cultural Vigour Relative to Grade.	Strain.	Virus.	Original Porcine Material.
III.	Below average	Original Material	P VIII.	Submaxillary lymphatic gland.
"	"	Original Material	P XXXII.	Submaxillary lymphatic gland.
"	Average	Original Material	P XXXIII.	Submaxillary lymphatic gland.
"	"	Original Material	P XXXIX.	Submaxillary lymphatic gland.
"	"	Original Material	P XLVII.	Submaxillary lymphatic gland.
"	"	Original Material	P LII.	Submaxillary lymphatic gland.
"	Above average	G. P. 1612 from Calf 282	P III.	Submaxillary lymphatic gland.
"	"	Rabbit 557 from Calf 312	P IV.	Submaxillary lymphatic gland.
"	"	Original Material	P XXXVII.	Submaxillary lymphatic gland.
"	"	Original Material	P XLII.	Submaxillary lymphatic gland.
"	"	Original Material	P XLVI.	Submaxillary lymphatic gland.
"	"	Original Material	P XLIX.	Submaxillary lymphatic gland.
IV.	Below average	Original Material	P XLI.	Submaxillary lymphatic gland.
"	Average	Pig. 166	"	" " "
V.	Below average	Original Material	P IV.	Submaxillary lymphatic gland.
"	"	Calf 312	"	" " "
"	Average	Pig 202	P XLI.	Submaxillary lymphatic gland.

CLASSIFICATION OF SWINE STRAINS.

(2) AVIAN.

Strain.	Virus.	Original Porcine Material.
Original Material	P II.	Submaxillary lymphatic gland.
Monkey 118 (Local Lesion)	"	" "
Original Material	P III.	Submaxillary lymphatic gland.
Original Material	P XXI.	Submaxillary lymphatic gland.
Original Material	P XXVII.	Submaxillary lymphatic gland.
Original Material	P XLIV.	Submaxillary lymphatic gland.

SUMMARY OF RESULTS.

58 swine viruses have been investigated. 51 of these were found to be identical culturally with ordinary bovine viruses, and are classed in one or other of Grades I, II, and III. These call for no further comment, but attention may be directed to the remaining 7 :—

P IV.

The strains from the original material of this virus and from Calf 312 grew abundantly and were classed in Grade V. But the strain from Rabbit 557, inoculated with culture from Calf 312, grew less well and was classed in Grade III ; and the growths from 2 strains of Rabbit 556, also inoculated with culture from Calf 312, were still poorer, and were classed in Grade II.

P XLI.

The strains from original material and Pig 166 were classed in Grade IV, the strain from Pig 202 in Grade V.

P II, P III, P XXI, P XXVII, P XLIV.

The strains from the original material of these 5 viruses presented the typical characters of the avian tubercle bacillus.

But the strain of P II from the spleen of Monkey 118, and a strain of P III derived from Calf 282 were mammalian in character and were classed in Grade III.

IV.—THE CULTURAL CHARACTERS OF EQUINE VIRUSES.

CULTURAL CHARACTERS OF INDIVIDUAL STRAINS.

VIRUS—E I.

(Lesions from a Horse.)

Material Received.—Primary culture, on egg, 26 days old, isolated from a guinea-pig which had been inoculated with lesions from a horse.

Cultural Characters.—For several generations the growth was extremely poor both on pure serum and glycerinated serum ; no growth could be obtained on either broth or potato, and on glycerin-agar there was no more than a faint grey haze. The culture was again tested when it had reached the 17th generation on serum. It then grew much better on serum and on other media. On broth the surface was covered in three weeks with a thin, semi-opaque pellicle which afterwards became slightly thicker and showed no tendency to sink. On glycerin-agar the greater part of the surface was covered in three weeks with a grey, somewhat dense layer ; no further increase took place. On potato a scanty, yellowish growth was slowly formed.

The bacilli did not exhibit much divergence from the ordinary serum type, but on potato, in a 56 days' film, beaded forms as long as 4 μ were fairly frequent.

CLASSIFICATION.

In the earlier generations the growths were poorer than those of any previously recorded culture in Grade I ; but after prolonged subculture on serum the growths on test media became about equal to the average growths in Grade III.

VIRUS—E II.

(Mesenteric Glands of a Horse.)

(1.)

Strain.—G.P. from ORIGINAL MATERIAL.

Material Received.—Primary culture, on egg, 26 days old, isolated from a guinea-pig inoculated with the original material.

Cultural Characters.—In the earlier generations growths were very poor both on pure serum and glycerinated serum ; on broth no growth could be obtained ; on glycerin-agar there was only a slight grey haze ; on potato growth was hardly visible to the naked eye, but under the microscope there was well marked evidence of growth, and the bacilli were longer (1 to 3 μ) than those grown on serum. The cultures were tested again when they had reached the 19th generation on serum. They then grew much better both on serum and on other media. On broth a thin, grey, opaque layer covered half the surface in a fortnight ; it showed some tendency to sink, but most of the pellicle survived and became thicker. On glycerin-agar a fairly dense, slightly granular layer was formed. On potato growth was readily obtained, though not very abundant, and exhibited yellow colonies.

CLASSIFICATION.

In the earlier generations, amongst the poorest growths of Grade I ; after frequently repeated subculture on serum, amongst the better growths of Grade III though not equal to the best.

(2.)

Strain.—CALF 404.

Material Received.—Fourteenth generation, 9 days old, of culture isolated from Calf 404.

Cultural Characters.—Good growths were obtained on serum. On broth a few small islands were formed. On glycerin-agar there was little more than a grey haze. On potato there was a scanty, grey growth.

CLASSIFICATION.

Amongst the average growths of Grade I.

(3.)

Strain.—CALF 504. (*Prescapular Gland.*)

Material Received.—Fifth generation, 9 days old, of culture isolated from the prescapular gland of Calf 504.

Cultural Characters.—Good growths were obtained on serum. On broth, glycerin-agar, and potato, cultures were identical with those of the preceding strain.

CLASSIFICATION.

Amongst the average growths of Grade I.

(4.)

Strain.—CALF 504. (*Lung.*)

Material Received.—Ninth generation, 170 days old, of culture isolated from the lungs of Calf 504.

Cultural Characters.—The growths on serum were at first poor, but afterwards improved On broth, glycerin-agar, and potato, the cultural characters resembled those of *Strain* (3).

CLASSIFICATION.

Amongst the average growths of Grade I.

(5.)

Strain.—CALF 506.

Material Received.—Fifteenth generation, 9 days old, of culture isolated from Calf 506.

Cultural Characters.—Good growths were obtained on serum. On broth growth commenced in the fourth week and produced a moderately thick, speckled pellicle which covered about half the surface ; some deposit was then formed, and the surface never became completely covered. On glycerin-agar the growth was scanty, amounting to slightly more than a grey haze. On potato there was a scanty, grey growth.

CLASSIFICATION.

Amongst the better growths of Grade I.

(6.)

Strain.—CALF 590.

Material Received.—Second generation, 13 days old, of culture isolated from Calf 590.

Cultural Characters.—Good growths were obtained on serum. On broth a few small islands were obtained. On glycerin-agar a thin, uniform, slightly opaque layer was formed. On potato growth was scanty and non-pigmented.

CLASSIFICATION.

Amongst the better growths of Grade I.

(7.)

Strain.—CALF 596.

Material Received.—Third generation, 20 days old, of culture isolated from Calf 596.

Cultural Characters.—Good growths were obtained on serum. On test media the cultural characters were found to be identical with those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

(8.)

Strain.—FOWL 398.

Material Received.—Primary culture, 81 days old, isolated from Fowl 398.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar the growth was poor and typically mammalian but was less scanty than that of the preceding strain.

CLASSIFICATION.

About the average of Grade II.

VIRUS—E III.

(Mesenteric Glands of a Horse.)

Material Received.—Primary culture, on egg, 26 days old, isolated from a guinea-pig which had been inoculated with the original material.

Cultural Characters.—In the earlier generations growth was very poor on serum; none was obtained on broth and nothing definite could be seen on glycerin-agar; on potato, though growth was hardly visible to the naked eye, there was well marked evidence of growth under the microscope. The culture was tested again when it had reached the 17th generation on serum. It then grew much better on serum and on other media. On broth the surface was covered in four weeks with a thin layer which gradually developed thicker patches and showed no tendency to sink. On glycerin-agar a moderately dense layer was produced with some raised, granular points. On potato growth was readily obtained but did not become abundant; it exhibited a yellow pigment.

The bacilli on media other than serum exhibited only a slight deviation from the serum type.

CLASSIFICATION.

In the earlier generations, amongst the poorest growths of Grade I; after frequently repeated subculture on serum, amongst the better growths of Grade III, though not equal to the best.

VIRUS—E IV.

(Spleen of a Horse.)

(1.)

Strain.—G.P. from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 35 days old, of culture isolated from a guinea-pig which had been inoculated with the original material.

Cultural Characters.—Growth on serum was fairly good. On broth the surface became about two-thirds covered in three weeks; the pellicle was grey and of unequal density; it thickened slightly and showed no tendency to sink. On glycerin-agar a dull grey, fairly thick layer was slowly formed. On potato there was a scanty, non-pigmented growth.

The bacilli grown on broth and glycerin-agar were only slightly longer than serum bacilli, but amongst them there were forms which were considerably thicker than the normal. On potato the bacilli were longer (·75 to 4 μ) and generally straight and uniformly stained.

CLASSIFICATION.

Amongst the better growths in Grade III.

(2.)

Strain.—G.P. 2420 from BABOON 72.

Material Received.—Fifth generation, 8 days old, isolated from G.P. 2420, which had been inoculated from Baboon 72.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered with a thin pellicle in five weeks; the growth then became moist and sank. On glycerin-agar a moderately thick grey layer with many large colonies was formed. On potato a moderate amount of grey growth was obtained.

CLASSIFICATION.

Amongst the better growths of Grade III.

(3.)

Strain.—BABOON 74.

Material Received.—Second generation, 5 days old, of culture isolated from Baboon 74.

Cultural Characters.—Good growths were obtained on serum. On broth the characters were the same as with *Strain* (2). On glycerin-agar there was a uniform, dull grey layer, not wrinkled, but showing very fine granules. On potato a grey, rather scanty growth was formed.

CLASSIFICATION.

Amongst the better growths of Grade III.

(4.)

Strain.—CALF 408.

Material Received.—Fifteenth generation, 15 days old, of culture isolated from Calf 408.

Cultural Characters.—Good growths were obtained on serum. On other media the cultural characters were identical with those of *Strain* (3).

CLASSIFICATION.

Amongst the better growths of Grade III.

(5.)

Strain.—CALF 498. (*Bronchial Gland.*)

Material Received.—Fifth generation, 57 days old, of culture isolated from a bronchial gland of Calf 498.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was covered with a thin pellicle in five weeks, and then the growth began to become moist and sink. On glycerin-agar a grey layer of unequal density was formed. On potato the growth was grey and not very copious.

CLASSIFICATION.

Amongst the average growths of Grade III.

(6.)

Strain.—CALF 498. (*Lungs.*)

Material Received.—Fourth generation, 14 days old, of culture isolated from the lungs of Calf 498.

Cultural Characters.—Good growths were obtained on serum. On broth the first cultures were very poor and readily sank ; later cultures covered the surface with a thin grey layer which showed less tendency to sink. On glycerin-agar a moderately thick, uniform layer was slowly formed, but showed no tendency to become wrinkled. On potato there was a thin grey layer.

CLASSIFICATION.

Equal to the average growths in Grade III but not equal to the best.

(7.)

Strain.—CALF 502.

Material Received.—Sixth generation, 18 days old, of culture isolated from Calf 502.

Cultural Characters.—Good growths were obtained on serum. On broth the surface was nearly covered in five weeks, and then the growth became moist and sank. On glycerin-agar a moderately good grey layer with several raised colonies was formed. On potato there was a moderately thick, grey layer.

CLASSIFICATION.

Amongst the average growths of Grade III.

(8.)

Strain.—CALF 616.

Material Received.—Third generation, 20 days old, of culture isolated from Calf 616.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar there was an unequal growth, thin on the whole, and failing to increase after the first fortnight.

CLASSIFICATION.

Amongst the best growths of Grade II.

(9.)

Strains.—FOWLS 438 and 440.

Material Received.—Primary cultures, 78 and 60 days old, isolated respectively from Fowls 438 and 440.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar the growths were typically mammalian and were identical with those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

(10.)

Strain.—MONKEY 178.

Material Received.—Primary culture, 38 days old, isolated from Monkey 178.

Cultural Characters.—Good growths were obtained on serum. On broth growth was slow, but most of the surface was covered at the end of two months with a thin layer. On glycerin-agar a grey layer of moderate but unequal thickness was formed; the surface was not well covered. On potato, a thin, cream-coloured layer was formed.

CLASSIFICATION.

Amongst the poorer growths of Grade III.

VIRUS—E V.

(1.)

(Spleen of Horse.)

Material Received.—Primary culture, 30 days old, isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. On broth the three first cultures failed; the fourth rapidly covered the surface with a thin layer which soon became moist and sank. On glycerin-agar, a thin, semi-opaque, uniform layer was formed. On potato there was a scanty, non-pigmented growth.

CLASSIFICATION.

Amongst the better growths of Grade II.

(2.)

(Lung of Horse.)

Material Received.—Primary culture, 30 days old, isolated from the original material.

Cultural Characters.—Good growths were obtained on serum. The cultures failed at first on broth, but afterwards grew like those of the preceding strain. On glycerin-agar and potato the cultures were identical with those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

CLASSIFICATION OF EQUINE VIRUSES.

Grade.	Cultural Vigour Relative to Grade.	Strain.	Virus.	Original Equine Material.
I.	Below average	G.P. from Original Material (early generations)	E I.	Lesions from a horse.
„	„	G.P. from Original Material (early generations)	E II.	Mesenteric gland.
„	„	G.P. from Original Material (early generations)	E III.	Mesenteric gland.
„	Average	Calf 404	E II.	Mesenteric gland.
„	„	Calf 504 (Prescapular Gland)	„	„
„	„	„ (Lung)	„	„
„	Above average	Calf 506	„	„
„	„	Calf 590	„	„
„	„	Calf 596	„	„
II.	Average	Fowl 398	„	„
„	Above average	Calf 616	E IV.	Spleen.
„	„	Fowl 438	„	„
„	„	Fowl 440	„	„
„	„	Original Material	E V.	Spleen.
„	„	Original Material	„	Lung.
III.	Below average	Monkey 178	E IV.	Spleen.
„	Average	G.P. from Original Material (later generations)	E I.	Lesions from a horse.
„	„	Calf 498	E IV.	Spleen.
„	„	„ (Lung)	„	„
„	„	Calf 502	„	„
„	Above average	G.P. from Original Material (later generations)	E II.	Mesenteric gland.
„	„	G.P. from Original Material (later generations)	E III.	Mesenteric gland.
„	„	G.P. from Original Material	E IV.	Spleen.
„	„	G.P. 2420 from Baboon 72...	„	„
„	„	Baboon 74	„	„
„	„	Calf 408	„	„

Summary of Results.

These five equine viruses resembled in cultural growth the ordinary bacillus isolated from the bovine. The first three grew very badly in early generations, presenting more difficulty in this respect than even very poorly growing bovine viruses; but after prolonged subculture they showed marked improvement, though not surpassing the maximum luxuriance of Grade III. Strains passed through other species of animals, including the fowl, showed no important changes in cultural characters.

V.—THE CULTURAL CHARACTERS OF VIRUSES ISOLATED FROM OTHER SPONTANEOUSLY AFFECTED MAMMALS.

CULTURAL CHARACTERS OF INDIVIDUAL STRAINS.

A.

VIRUS—F I.

(Mesenteric Gland of Cat.)

Material Received.— Sixth generation, 20 days old, of culture isolated from original material (the mesenteric gland of a cat).

Cultural Characters.—Good growths were obtained on serum. On broth a thin grey pellicle was formed ; it covered the greater part of the surface in a month and then showed a tendency to sink ; in another flask a thin pellicle covered the surface and remained floating at the end of two months. On glycerin-agar growth commenced very slowly during the first fortnight but continued for some weeks afterwards, and at the end of the second month there was a moderately thick layer with some raised colonies but no wrinkles. On potato a thin, cream-coloured layer was formed.

CLASSIFICATION.

Rather better than the average growths of Grade III but not equal to the best.

B.

VIRUS—Z I.

(Lung of Gnu.)

Material Received.—Primary culture, 23 days old, isolated from the lung of a gnu.

Cultural Characters.—Good growths were readily obtained on pure serum. On broth a very dense layer had spread over two-thirds of the surface in a fortnight, and in the following week the surface was completely covered ; the pellicle was particularly dense and wrinkled. On glycerin-agar the growth was rapid, very dense, very wrinkled, and warty. On potato a brown, fairly abundant, heaped up growth was readily formed.

CLASSIFICATION.

One of the most luxuriant growths in Grade V.

C.

VIRUS—Z III.

(Lung of Antelope.)

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 49 days old, isolated from the lung of an antelope.

Cultural Characters.—Growths on pure serum were satisfactory. Good growths were obtained on glycerinated serum. On broth the surface became covered in five weeks with a tough pellicle of unequal density which showed no tendency to sink. On glycerin-agar fairly thick, granular growths were obtained ; several cultures were grown, and although some yielded better growths than others none could be described as distinctly luxuriant. On potato growths were readily obtained but did not become very abundant ; they exhibited a brownish yellow pigment.

On media other than serum the bacilli were longer and less regularly stained than serum grown bacilli.

CLASSIFICATION.

One of the poorest growths of Grade IV.

(2.)

Strain.—RABBIT 1041.

Material Received.—Second generation, 14 days old, of culture isolated from Rabbit 1041.

Cultural Characters.—Good growths were obtained on serum. On broth, the surface became slowly covered with a moderately thick and tough layer showing no tendency to sink. On glycerin-agar there was a thick, granular, and very wrinkled layer. On potato the growth was moderate in amount and coloured with brown pigment.

CLASSIFICATION.

An average growth of Grade IV.

(3.)

Strain.—RABBIT 1241.

Material Received.—Primary culture, 103 days old, isolated from Rabbit 1241, which was inoculated with original material.

Cultural Characters.—Good growths were obtained on serum. On broth only a few patches of growth were obtained in the first culture, but in a second the greater part of the surface was covered in two months with thick patches. On glycerin-agar a thick layer was readily formed, and afterwards became wrinkled and showed a few warty elevations. On potato a moderate amount of brick-red growth was obtained.

CLASSIFICATION.

Equal, on the whole, to the average of Grade IV.

(4.)

Strain.—G.P. 2733 from RABBIT 1241.

Material Received.—Second generation, 20 days old, of culture isolated from G.P. 2733, which was inoculated from Rabbit 1241.

Cultural Characters.—Good growths were obtained on serum. On broth the first culture only produced a few islands; the second covered the greater part of the surface in two months with a moderately thick layer. On glycerin-agar and potato the growths were identical with those of the preceding strain.

CLASSIFICATION.

Identical with the preceding strain.

(5.)

Strain.—RABBIT 1368.

Material Received.—Fourth generation, 8 days old, isolated from Rabbit 1368.

Cultural Characters.—Good growths were obtained on serum. On broth the first culture was poor; the second became covered with a thick warty pellicle during the second month. On glycerin-agar and potato the growths were identical with those of the two preceding strains.

CLASSIFICATION.

Nearly identical with the two preceding strains.

CLASSIFICATION OF VIRUSES ISOLATED FROM OTHER SPONTANEOUSLY AFFECTED MAMMALS.

Grade.	Cultural Vigour Relative to Grade.	Strain.	Virus.	Original Material.
III.	Above average	Original Material	F I.	Mesenteric gland of a Cat.
IV.	Below average	Original Material	Z III.	Lung of an Antelope.
„	Average	Rabbit 1041	„	„
„	„	Rabbit 1241	„	„
„	„	G.P. 2733 from Rabbit 1241 ...	„	„
„	„	Rabbit 1368	„	„
V.	Above average	Original Material	Z I.	Lung of a Gnu.

Summary of Results.

The virus from the cat grew fairly well, but not better than some bovine viruses. The virus from the antelope grew as well as many slightly virulent human viruses; and the virus from the gnu was extremely luxuriant in its growth.

VI.—THE CULTURAL CHARACTERS OF BACILLI ISOLATED FROM BIRDS.

CULTURAL CHARACTERS OF INDIVIDUAL STRAINS.

VIRUS—A I.

(Intestinal Lesions of Touracon.)

Material Received.—Primary culture, 67 days old, isolated from the intestinal lesions of a touracon.

Cultural Characters.—A scanty growth was obtained on pure serum; on glycerinated serum the growth was abundant; on both media it was of a slimy character. No surface growth was obtained on broth. On glycerin-agar a white, slimy growth developed and attained its maximum in about a fortnight. On potato the growth slowly increased and gradually developed pigment; in two months it consisted of abundant, raised, yellow colonies.

On horse serum, in a 15 days' film, the bacilli varied in length from ·5 to 1·25 and occasionally 1·5 μ; they were straight and uniformly stained. On glycerinated horse serum some longer and some thicker forms were observed. On glycerin-agar, in a 42 days' film, the majority of the bacilli varied in length from 1 to 3 μ and were straight and uniformly stained; much longer (4 to 8 μ) and thicker forms were also present; some of these exhibited club-shaped extremities. On potato, in a 56 days' film, the bacilli varied in length from ·75 to 3 μ; the majority were straight and beaded.

Classification

A typical avian tubercle bacillus.

VIRUS—A II.

(Spleen of Pigeon.)

Material Received.—Third generation, 5 days old, of culture isolated from the spleen of a pigeon.

Cultural Characters.—Growth on pure serum was very poor; on glycerinated serum an abundant slimy growth was obtained. On broth, with the exception of a few very small islands, there was no surface growth. On glycerin-agar a white, slimy growth was readily obtained; no increase was observed after the second week. On potato the growth was slow, but gradually became fairly copious and developed a yellow pigment; growth continued for about seven weeks.

On horse serum, in a 15 days' film, the bacilli varied in length from ·5 to 1·5 μ, and were straight and uniformly stained. On glycerinated serum some longer (2 to 3 μ) and thicker forms were found. On glycerin-agar, in a 42 days' film, the majority of the bacilli were no longer than those grown on glycerinated serum, but stained more irregularly; much longer (4 to 6 μ), thicker, and irregular forms were also present. On potato, in a 56 days' film, the bacilli varied in length from 1 to 4 μ; they were straight, or only slightly curved, and generally beaded.

Classification.

A typical avian tubercle bacillus.

VIRUS—A III.

(Lung of Crane.)

Material Received.—Second generation, 40 days old, of culture isolated from the lung of a crane.

Cultural Characters.—A moderate amount of slimy growth was obtained on pure serum, but the growth on glycerinated serum was much better and was of the typical slimy character. On broth no surface growth was obtained. On glycerin-agar the usual white, slimy growth readily appeared, and attained its maximum development about the end of the second week. On potato growth continued for about seven weeks and became abundant and cream-coloured.

On horse serum, in a 15 days' film, the bacilli varied in length from ·5 to 1·25 μ and were straight and uniformly stained. On glycerinated horse serum the bacilli were also notably short and uniform in character, resembling those grown on pure serum. On glycerin-agar, in a 42 days' film, most of the bacilli resembled those grown on serum, but were a little longer; a few much longer (4 to 5 μ) and thicker forms were also present. On potato, in a 56 days' film, the bacilli varied in length from 1 to 4·5 μ, and were generally straight, or only slightly curved, and beaded.

Classification.

A typical avian tubercle bacillus.

VIRUS—A IV.

(Liver of Pheasant I.)

(1.)

Strain.—Original Material.

Material Received.—Primary culture, 19 days old, isolated from the liver of Pheasant I.

Cultural Characters.—Some growth, of a slimy nature, was obtained on pure serum; on glycerinated serum an abundant slimy growth was obtained. No surface growth was obtained on broth. On glycerin-agar the usual white slimy growth, taking about a fortnight to fully develop, was obtained. On potato growth continued for about seven weeks and gradually became cream-coloured; the yield was fairly abundant.

On horse serum, in a 15 days' film, the bacilli varied in length from about ·75 to 1·5 or 2 μ and were straight and uniformly stained. On glycerinated serum some longer and thicker forms were found. On glycerin-agar, in a 42 days' film, the bacilli resembled those grown on glycerinated serum; long, thick bacilli were present but rare. On potato, in a 56 days' film, the bacilli varied in length from ·75 to 4 μ and were for the most part straight and beaded.

CLASSIFICATION.

A typical avian tubercle bacillus.

(2.)

Strain.—CALF 456.

Material Received.—Third generation, 7 days old, of culture isolated from the prescapular gland of Calf 456.

Cultural Characters.—Good growths were obtained on serum. On broth a thin pellicle was rapidly produced but became moist during the third week and then rapidly sank. On glycerin-agar an opaque, dry, dull grey layer of moderate density was produced. On potato there was a thin, grey layer beset with pinpoint colonies; these colonies became larger towards the end of the second month and assumed a yellowish pigment.

On media other than serum the bacilli varied in length from ·75 to 3 μ, and were for the most part straight and uniformly stained.

CLASSIFICATION.

The cultures resemble those of mammalian bacilli classed amongst the poorer growths of Grade III

(3.)

Strain.—CALF 494. (*Local Lesion.*)

Material Received.—Second generation, 36 days old, of culture isolated from the local lesion of Calf 494.

Cultural Characters.—Good growths of a slimy appearance, typical of the avian bacillus, were obtained on glycerinated serum. On broth the bacilli refused to form a surface growth. On glycerin-agar and on potato typical thick, slimy growths were obtained; the yield on potato was of a light brown colour.

The bacilli grown on glycerin-agar varied in length from 1·5 to 4 μ and stained very irregularly; long, thickened forms occurred frequently.

CLASSIFICATION.

A typical avian bacillus.

(4.)

Strain.—CALF 494. (*Prescapular Gland.*)

Material Received.—Third generation, 140 days old, of culture isolated from the right prescapular gland of Calf 494.

Cultural Characters.—Good growths were obtained on glycerinated serum. On broth the bacilli refused to form a surface pellicle. On glycerin-agar and on potato good growths, typical of the avian bacillus, were obtained.

CLASSIFICATION.

A typical avian bacillus.

(5.)

Strain.—FOWL 298 (*Local Lesion*) from CALF 456.

Material Received.—Primary culture, 51 days old, isolated from the local lesion of Fowl 298, which had been inoculated from Calf 456.

Cultural Characters.—Growths on glycerinated serum were good and rather moist, though not slimy. Growths on pure serum were very poor at first, but afterwards improved. On broth a thin, grey layer, speckled with denser colonies gradually spread over the surface. On glycerin-agar the growth was dry, grey, thin on the whole, but contained a few warty colonies. On potato a dry, white, and scanty layer was slowly formed.

On broth and potato the bacilli varied in length from 2 to 6 μ, and on glycerin-agar from 1·5 to 3 μ. Many forms were beaded but no other irregularities of staining were noted

CLASSIFICATION.

Mammalian: amongst the poorer growths of Grade III.

(6.)

Strain.—FOWL 298. (*Liver.*)

Material Received.—Primary culture, 51 days old, isolated from the liver of Fowl 298.

Cultural Characters.—On pure serum very scanty; on glycerinated serum abundant and slimy. No growth was obtained on broth. On glycerin-agar the typical white slimy growth was obtained. On potato there was a typical yellow, slimy growth.

On potato and glycerin-agar the bacilli showed only slight morphological deviations from the serum type.

CLASSIFICATION.

A typical avian bacillus.

VIRUS—A V.

(Spleen of Pheasant II.)

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Third generation, 10 days old, isolated from culture of the spleen of Pheasant II.

Cultural Characters.—On pure serum growth was extremely scanty; on glycerinated serum growths were good and were of the typical slimy appearance. The culture was kept up on the latter medium. On broth no surface growth was obtained. On glycerin-agar a thick, white slimy growth developed and attained its maximum during the first fortnight. On potato growth progressed for about two months, became fairly abundant, and gradually assumed a yellowish colour.

On glycerinated serum and glycerin-agar, in addition to forms resembling ordinary serum bacilli, longer (2 to 3·5 μ) and much thicker forms were present. These thicker forms were not noted in the potato growth.

CLASSIFICATION.

A typical avian bacillus.

(2.)

Strain.—G. P. 2531.

Material Received.—Primary culture, 103 days old, isolated from G. P. 2531, which had been inoculated with the contents of a collodion capsule.

Cultural Characters.—On pure serum growth was very scanty; on glycerinated serum growth was good, not slimy but forming a tough, sticky paste. Subcultures on the test media were made from glycerinated serum, as the subcultures attempted from the very scanty growth on pure serum yielded nothing. On broth the surface became gradually covered with a thin, grey pellicle, speckled with small patches of denser material. On glycerin-agar the growth was dry, grey, rather thin, but interspersed with colonies and patches of denser material. On potato the surface became slowly covered with a dry, thin, cream-coloured layer.

On potato, in a 56 days' film, the bacilli varied in length from 2 to 6 μ and were mostly curved and beaded. On broth, in a 28 days' film, they were also long (2 to 5 μ), but more uniformly stained. On glycerin-agar, in a 45 days' film, they were shorter (1 to 3 μ), and about half were straight and uniformly stained.

CLASSIFICATION.

The characters, on the test media, are those of mammalian bacilli equivalent to the average growths of Grade III.

VIRUS—A VI.

(Spleen of Pheasant III.)

Material Received.—Seventh generation, 20 days old, of culture isolated from the spleen of Pheasant III.

Cultural Characters.—Good growths were obtained on glycerinated serum, and were of the slimy character topical of the avian bacillus. On broth no surface growth was obtained. On glycerin-agar the growth was white and slimy but not quite so abundant as with some viruses. On potato the growth was slimy and abundant, and exhibited a brown pigmentation.

On all media the majority of the bacilli were very short but a smaller number of long, thickened forms occurred.

CLASSIFICATION.

A typical avian bacillus.

VIRUS—A VII.

(Spleen of Fowl A.)

Material Received.—Primary culture, 22 days old, isolated from the spleen of Fowl A.

Cultural Characters.—Good growths, of a typical slimy appearance, were obtained on glycerinated serum. On broth the bacilli refused to form a surface growth. On glycerin-agar a typical white, slimy growth was formed. On potato a moist, yellow growth was slowly produced and became fairly abundant.

CLASSIFICATION.

A typical avian bacillus.

VIRUS—A VIII.

(Spleen of Fowl B.)

Material Received.—Second generation, 11 days old, of culture from spleen of Fowl B.

Cultural Characters.—Good growths were obtained on glycerinated serum. On broth no surface growth took place but the bacilli grew at the bottom of the flask. On glycerin-agar and potato the growths were rather poor at first but good in later generations. All the growths showed the typical slimy characters of the avian bacillus.

CLASSIFICATION.

A typical avian bacillus.

VIRUS—A X.

(Fowl D.)

(1.)

Strain.—RABBIT from ORIGINAL MATERIAL.

Material Received.—Third generation, 30 days old, of culture isolated from a rabbit inoculated with Fowl D.

Cultural Characters.—Good growths were obtained on glycerinated serum. No surface growth was produced on broth. On glycerin-agar and potato abundant, slimy growths were readily formed, the growth on potato being coloured with a brownish yellow pigment.

CLASSIFICATION.

A typical avian bacillus.

(2.)

Strain.—RABBIT 1866 FROM CALF 574.

Material Received.—Primary culture, 43 days old, isolated from Rabbit 1866, which had been inoculated with culture from a bronchial gland of Calf 574.

Cultural Characters.—Good growths were obtained on serum. On glycerin-agar the growth was mammalian in type and formed a thin grey layer.

CLASSIFICATION.

About the average of Grade II.

CLASSIFICATION OF VIRUSES ISOLATED FROM BIRDS.

(1.) AVIAN TYPE.

Strain.	Virus.	Original Avian Material.
riginal Material	A I.	Intestinal nodule of a Touracon.
Original Material	A II.	Spleen of Pigeon.
Original Material	A III.	Lung of Crane.
Original Material	A IV.	Liver of Pheasant I.
Calf 494 (Local Lesion)	„	„ „
„ (Prescapular Gland)	„	„ „
Fowl 298 (Liver)	„	„ „
Original Material	A V.	Spleen of Pheasant II.
Original Material	A VI.	Spleen of Pheasant III.
Original Material	A VII.	Spleen of Fowl A.
Original Material	A VIII.	Spleen of Fowl B.
Rabbit inoculated with Original Material ...	A X.	Fowl D.

(2.) MAMMALIAN TYPE.

Grade.	Cultural Vigour Relative to Grade.	Strain.	Virus.	Original Avian Material.
II.	Average	Rabbit 1866 from Calf 574... ...	A X.	Fowl D.
III.	Below average	Calf 456	A IV.	Liver of Pheasant I.
„	„	Fowl 298 (Local Lesion) from Calf 456.	„	„ „
„	Average	G.P. 2531	A V.	Spleen of Pheasant II.

Summary of Results.

All the cultures raised from original avian material were practcially identical, presenting in common the characteristics of :—very poor growth on pure serum ; copious growth on glycerinated serum ; refusal to spread from a sloping surface of glycerinated serum on to the surface of broth, but the formation of powdery growth at the bottom of the broth ; copious, pale, slimy growth on glycerin-agar ; abundant, pigmented growth on potato ; in films examined microscopically, a free separation of the bacilli (to be contrasted with the glueing together of mammalian bacilli), a marked prevalence of very short forms in some cultures, and a marked pleomorphism, with not infrequent branching, in others.

Four strains, recovered after residence of the bacilli in mammals, presented the mammalian characters of Grades II and III.

PART IV.

COMPARISON OF MAMMALIAN WITH AVIAN VIRUSES.

I.—THE CULTURAL AND MICROSCOPIC CHARACTERISTICS OF THE MAMMALIAN BACILLI.

The starting-point of my culture work has nearly always been a culture isolated by one or other of my colleagues either upon pure inspissated blood-serum or upon Dorset's egg medium.

For the purpose of keeping a strain alive and in vigorous condition, without altering its characteristics, I have found pure inspissated blood-serum to be the best medium for subculture. I have invariably kept up my stock on this medium, and when investigating differences of growth on various media, such media have always been inoculated from a serum tube. In order to bring out differences in cultural luxuriance, I have constantly employed three media, *viz.*, glycerinated broth, glycerin-agar, and glycerinated potato. I have also used in many instances glycerin-serum, Lemco-peptone broth with the addition of some sugar, starch, alcohol, or glucoside, and an asparagin medium containing some similar addition.

I have usually prepared the serum, which has been sterile, by exposing it in slanted test-tubes and in an atmosphere saturated with moisture, to a temperature gradually raised up to 75° C.; it has been kept at this temperature until firm coagulation has taken place. The serum from different animals varies in its nutritive qualities. I have found that the serum from adult bovines is invariably good; calf serum and horse serum, though somewhat inferior, have proved quite good enough to promote a satisfactory growth for subcultures. The dog serum which I have used has generally been very good; I have also obtained good growths on pig serum. For most of my serum I am indebted to my colleagues.

For the preparation of glycerinated serum, broth, agar, and potato I have used 5 per cent. of pure glycerin. In other respects the broth, agar, and potato were prepared in the ordinary way, the acidity of the broth to phenolphthaleine being from + 12 to + 16 per litre.

To cultivate the tubercle bacillus on broth I have devised a method which, so far as I know, is new. A layer of serum is inspissated on one of the narrow sides of an ordinary medicine flask. During the process of coagulation, the medicine flask, instead of resting upright on its narrow side, is tilted at a slight angle from the perpendicular, in order to give the serum a suitable slope. After cooling, a convenient amount of broth is poured into the flask, which is then laid on the broad side corresponding to the broad end of the wedge of the serum. The serum slope above the level of the broth is inoculated in the usual way. The bacilli grow down the serum slope, and when they reach the broth, have the opportunity of growing over its surface. This seems to me a more reliable method than any other I know for objectively estimating the relative readiness or reluctance of different strains of tubercle bacilli to grow on a liquid medium.

All culture tubes and broth flasks are plugged with cotton wool which is sealed with paraffin as soon as sterilisation has been completed.

The temperature for incubation has been 37½° C.

With regard to primary cultures I quote the following examples of cultures raised in my laboratory, the infected tissues having been provided for me by my colleagues.

Taking a typical bovine virus, B XXIV, I raised cultures from the spleen of a guinea-pig inoculated with the original bovine material. In the portion of the spleen selected, bacilli were somewhat scanty; a smear preparation revealed from two to six in every five or six microscopic fields. The bacilli were from 2 to 3 μ in length and most of them were curved and beaded. After fragments of this material had been rubbed with the platinum loop over the surface of dog serum tubes, minute grey colonies, dotted all over the surface, were distinctly visible in nine days. The colonies increased in size during the second and third weeks, and then development ceased. The ultimate yield consisted of a granular growth covering the entire surface and composed of discrete colonies which were closely set together and averaged from ¼ to ½ a millimeter in diameter. Four tubes of pure bovine serum were also inoculated with a broken-up portion of the same spleen. In all of them, as is usual with bovine serum but not with dog serum, a scum formed over the surface before any growth appeared. In one of the tubes colonies were just visible, emerging from the scum, but not so numerous as on the dog serum tubes, in nine days. The other three tubes showed no growth, visible to the naked eye, in twelve days; they were then rubbed up with the platinum loop, and two days afterwards a definite growth appeared all over the surface in all of them; at the end of three weeks a fairly thick layer was obtained, quite as copious as on any of the dog serum tubes. On glycerinated bovine serum my attempts to raise primary cultures failed.

The human virus H 59. "L.B." proved to be identical with the last-mentioned bovine virus both as regards virulence and as regards scantiness of growth in subculture on test media. From the spleen and a sternal gland of a guinea-pig inoculated with the original human material of H 59. "L.B." I inoculated tubes of egg, pure bovine serum, and glycerinated bovine serum. On the egg medium the surface became well covered with minute points of growth in about three weeks, and on the glycerinated bovine serum several discrete white colonies were formed in about three weeks; but on the pure bovine serum no growth was obtained.

In the case of a swine virus, P XIV, which was found to be identical in every respect with an ordinary bovine virus, I obtained the sternal gland of a guinea-pig inoculated with the original material, and from this gland inoculated egg, pure dog serum, and glycerinated serum. The egg and the pure serum tubes yielded numerous minute colonies which became visible at the end of three weeks, but no growth was obtained on the glycerinated serum.

As another example I take the less virulent, and culturally more luxuriant, human virus H. 52. "T.F." I made cultures from the spleen of a guinea-pig killed fourteen days after inoculation with the human lung. A smear from the spleen showed that the bacilli were moderately numerous, and that they varied in length from 2 to 6 μ. On pure bovine serum growth was first definitely visible in eight days. At the end of a fortnight the surface was well covered with minute, semi-translucent colonies which were closely set together and gave the surface the appearance of round-glass. Growth increased until the end of the rst month, when the surface was well covered with a moderately dense, opaque layer, containing several raised, white colonies. Three tubes of glycerinated bovine serum were also inoculated with emulsion from he same spleen. In one, very minute colonies were ust visible about the eleventh or twelfth day; growth was then rapid, and at the end of a fortnight was more abundant than on pure serum, and whiter; at he end of a month there was a dense, continuous ayer of growth all over the surface, with large, raised white colonies. In the two other tubes inoculated, no colonies were visible until between the second and third weeks; at the end of a month, in one of these tubes the surface was covered with small white colonies which were nearly coalescent over most of the surface, and in some patches were heaped up; the other tube, at the same date, exhibited several large (up to 2 mm.), discrete colonies, the rest of the surface being bare.

The following is a general account of my observations on the growth of the mammalian bacillus in subculture.

On serum the surface becomes covered, generally within a fortnight, with a grey layer which is at first uniform in density but afterwards exhibits raised, dry, warty colonies. From some sera, particularly from the serum of adult bovines, the bacillus is often able to extract a yellow pigment. This capacity is, as a rule, confined to those strains of bacilli which grow luxuriantly on media other than serum, and with these more abundantly growing strains the addition of glycerin to the serum increases the amount of growth.

My method of observing the readiness or reluctance of a culture to transfer itself from serum to broth has brought out marked differences between the different viruses. In some cases, after there was a good layer of growth on the serum, a delay of a week or two, or even longer, took place before there was any growth on the broth; and then the formation of a pellicle often began as small, isolated islands, which gradually increased in area and coalesced. In other cases, fair-sized patches of pellicle commenced to form as soon as the culture on the layer of serum touched the margin of the broth; and often a continuous layer of growth was seen streaming down the serum slope and spreading uninterruptedly across the surface of the broth. The character of the pellicle, its rate of increase, and its ultimate yield also varied with different viruses. Some grew slowly, with the formation of a very thin, semi-translucent pellicle which was sometimes homogeneous, sometimes speckled with a few small white patches, but never produced a copious or uniformly thick growth. In the case of a few of these reluctantly growing viruses the patch of pellicle first formed was uniformly thick, but, as it extended, it thinned out, and it never became uniformly dense. In the case of the more rapidly growing viruses the pellicle sometimes was at first thin, but afterwards became much denser; frequently a dense pellicle was formed from the commencement. Marked differences have also been noted in the floating capacities of the pellicles. With some strains the growth was rapid and moderately thick, but soon became moist and then sank to the bottom. This tendency to sink readily was not exhibited by the dense, tough, and wrinkled types of pellicle.

Very marked cultural differences have also been brought out by the use of glycerin-agar. The following is a brief outline of the different types of growth which have been observed. (1) During the first five or six weeks the growth consisted of no more than a fine grey haze on the surface; towards the end of the second month a few large, isolated colonies might slowly form, originating, apparently, from the relatively larger particles of material originally inoculated. (2) An opaque, scanty, rather dull, grey layer was formed at the end of three weeks, but failed to make much progress subsequently, the growth always remaining thin and generally patchy. (3) The surface was completely covered with a somewhat dense, grey layer during the first fortnight; growth then continued, somewhat slowly, until about the sixth week; the total yield was moderately dense, sometimes smooth and dull, and sometimes with a brittle, granular appearance on the surface. (4) Growth was rapid and spread as a uniform, thick layer all over the surface. After about a fortnight the layer of growth became wrinkled and the wrinkles increased in prominence as the growth continued. (5) Growth still more abundant; the layer being both wrinkled and thrown into warty elevations.

On glycerin-potato marked differences were brought out which corresponded in a general way to the differences demonstrable on glycerin-agar. The types of growth may be, roughly, grouped into three: (1) At the end of about five weeks there was so little to be seen that it was doubtful if there had been any increase of the material inoculated. (2) At the end of the same period there was a clearly recognisable, though scanty, increase, without any pigment formation. (3) In from one to two weeks' time yellow colonies appeared; copious increase then took place, and in five weeks the cultures had already, sometimes, assumed a typical brownish-yellow, pulled bread appearance. As on glycerin-agar, the microscopic appearances of the bacilli varied considerably and exhibited no invariable relationship to the nature or amount of growth.

In Lemco-peptone broth I found glucose a fairly good substitute for glycerin. Two other sugars of the sixth series, galactose and fructose also favoured the growth of the bacillus, but not so markedly as glucose. Compound sugars were found to be very distinctly less useful. Probably the bacillus is unable to break up either these compounds or the glucosides; but salicin, which contains a phenol element, seemed to have a distinctly inhibitory effect. Of the alcohols, glycerin seems to stand alone in its beneficial effect; mannite, which is closely allied to it, produced no such influence, nor did dulcite, which is closely related to the sugars of the sixth series. Of the starches, dextrin seemed to exert a slightly favourable influence. Similar results were obtained when the above-mentioned substances were added to an asparagin medium in place of the Lemco broth.

My observations on the microscopic characters of the bacilli may be summarised as follows:—

Bacilli grown on pure serum, whether from the horse, bovine, pig, or dog, are notably uniform in character. They are usually straight and uniformly stained; their average length is about 1 μ, sometimes rather less and sometimes rather more. Forms at least as short as 0·75 μ are always present, and frequently there are a relatively small number of organisms in the film which are longer than the average, measuring from 1·5 to 2·5 μ. It is impossible from a microscopic examination of bacilli grown on serum to draw any inference as to their capacity for growth on other media.

When grown on glycerinated media the bacilli generally show a divergence from the serum type. Their average length is greater; they are frequently curved; and many of them exhibit irregularities of staining, the commonest irregularities being unequal distribution of the staining material, either in the form of regular beads or as one or more darkly stained rods or globules which may be found in any part of the length of the bacillus. These darkly stained rods or globules often show a tendency to split longitudinally, leading to the formation of new bacilli. In these irregular growths there are marked differences in the lengths of individual bacilli. In the same film there are generally to be found short forms (about 1 μ) and many much longer forms (2–4 μ); and in some specimens occasional still longer forms (5 to 7 or 8 μ) are present. Branched forms, in cultures grown on the media which I have employed, are extremely rare.

Bacilli obtained from living tissues are longer and less uniform in appearance than bacilli cultivated on pure serum. It is also observable that bacilli which are growing freely in the tissues of their host are usually, but not without exceptions, shorter and more uniformly stained than bacilli which have only succeeded in growing in scanty numbers. But though this tendency is recognisable, the virulence of tissue grown bacilli cannot be diagnosed with any certainty from their morphological appearances.

II.—THE HISTOLOGICAL CHARACTERISTICS OF INFECTIONS WITH THE MAMMALIAN VIRUSES.

A.—In Mammals.

In the Appendix to the Second Interim Report and in the preceding pages of the present volume I have reported my histological observations on the tissues infected with mammalian bacilli which have been sent to me for examination during the seven years 1903–9. These data present certain features which may be recorded independently of opinions derived from other sources regarding the histology of tuberculosis.

The tissues which I have examined reveal certain ways in which the bacillus has manifested its capacity for attacking the animal body. In many instances, excluding cases where the bacilli have been inoculated by the intravenous method, I have found that at an early period after introduction bacilli are present in tissues remote alike from the site of introduction and from the lymphatic glands draining that site. In some of the cases of early dissemination where the inoculation has been made by the subcutaneous method, I have examined the local lesion produced by the inoculation, and have there found bacilli in contact with, entering, and within dilated blood and lymph capillaries. In addition to such instances I have also found indirect evidence pointing to early dissemination. I have examined numerous cases where, at a longer period after infection, the total amount of disease has been slight, but disseminated foci have been present. Histological examination of such foci has generally proved them to be of a very definitely retrogressive character, and not of recent formation; and the lesions in the site of the body where the bacilli were introduced have also been found in a retrogressive condition. In such cases these disseminated foci appear to be attributable to an early dissemination of bacilli. Regarding the circumstances favouring early dissemination, a histological feature of much significance is that in early lesions there is often found an inflammatory stage, prior to the stage of circumscribed tubercle formation, and that the histological changes of the tissue in this early stage render possible the direct access of bacilli to the blood and lymph channels. As to the quantity of bacilli which may escape from such early lesions, my observations indicate that, with the exception of very acute and rapidly breaking down lesions, the number of bacilli which travel at once to distant parts is relatively small. And the many cases where, after sufficient lapse of time to allow tubercles to develop, the tissues remote from the primary focus have been found normal under the microscope, as well as to the naked eye, afford evidence that such early dissemination may either not occur at all or not in any appreciable amount.

Though some few bacilli may make an early escape, it is characteristic of the specimens which I have examined that, in whatever part of the body the bacilli may arrive, the tissues almost immediately set up obstacles to their further progress. In the local lesions produced by subcutaneous inoculation the mass of bacilli is at once surrounded by a barrier of wandering cells, and at later stages there is a more or less dense peripheral zone of fibrous tissue. The amount of resistance offered to the escape of bacilli from the site of inoculation varies with the degree of virulence of the bacilli inoculated. When the bacilli are of low virulence the barrier to further dissemination usually becomes impenetrable after a few weeks; but if they are of high virulence the barrier never becomes completely effective when the dose is large; when it is smaller, the amount of local resistance offered is variable, being very effective in some cases, but in others relatively slight. When dead bacilli are inoculated, though some of them may be demonstrable for three months or more in the local lesion, the subcutaneous resistance appears to be much greater than that offered to living bacilli of only slight virulence; and after cutaneous inoculations with highly virulent living bacilli there is very little evidence of dissemination.

The majority of the bacilli which escape from an infected area find their way into the neighbouring lymphatic glands, and the histology of glands thus infected shows that these tissues are of particularly high importance in affording obstacles to the further dissemination of bacilli. Early arrivals are generally detained either in the connective tissue where the afferent vessels open into the peripheral lymph sinus, or in the cellular framework of this lymph sinus, or amongst the lymphoid cells in the peripheral part of the cortex. The early period of detention is soon followed by tubercle formation, and then the facilities for escape of bacilli are small except in rapidly breaking down or in highly vascularised lesions.

The study of infected spleens shows that this organ plays a part in the detention of bacilli which is similar in many respects to that of the lymphatic glands, but owing to its rich blood supply and open vascular system the facilities for the escape of bacilli are greater, both in the early stages after their arrival and in lesions which are progressive and breaking down. The bone marrow is another important site where bacilli arrive at an early stage of infection and are detained for a variable period.

In the blood or lymph channels I have observed that obstruction of a vessel may arise from a clump of bacilli or a fragment of a detached or broken-down tubercle; bacilli which subsequently passed along such a path would be stopped at the obstructive focus. But much the most important agent which arrests the circulating bacillus is the endothelial cell. It is highly characteristic of the tubercle bacillus that it sticks to the cellular material with which it comes into contact, and I find the usual fate of a circulating bacillus is that it becomes adherent to the endothelial wall of a blood or lymph channel. Then follow cellular changes which may cause a projection into the lumen of a vessel, but which more frequently form a lesion on the external aspect of a capillary channel; in either case the primary result of the tubercular process is to form a cellular barrier detaining the bacillus *in situ*.

In the course of my investigations I have met with numerous instances, particularly in lesions which I have termed "retrogressive" or "non-progressive," where the barrier set up by the tissues against the further dissemination of bacilli appears to be effective, *i.e.*, the histological evidence indicates that, in the absence of some fresh exciting cause, the bacilli present are not likely to pass beyond the situation in which they are found. In other cases, again, particularly in lesions which I have termed "progressive" or "not definitely retrogressive," there is evidence of conditions favouring the further dissemination of bacilli from sites where they have been temporarily arrested.

In addition to the local dissemination which takes place in progressive tubercles where bacilli multiply and grow towards the periphery, the progressive escape of bacilli may be facilitated by various causes. There may be a local hyperaemia of the affected tissue, due, for example, to the absorption of irritant material or to an inoculation of tuberculin. This condition of hyperaemia I have often found in advanced lesions as well as in early stages of the disease, and where bacilli are numerous the chances are considerable that some of them may be carried away in the blood-stream. Then I have observed a proliferative stage of tubercle formation, associated with lesions not very acute in type but often containing bacilli in considerable numbers; in this condition opportunities arise for the escape of bacilli into newly formed capillary channels. But by far the most important mode of progressive dissemination found in my specimens is due to the penetration of tubercular foci into patent blood and lymph channels. In specimens properly fixed so as to show the red blood-corpuscles, dilated capillaries, containing intact corpuscles, are very frequently found in the midst of caseous areas; the slender capillary wall must sooner or later give way, and in favourable specimens the process of breaking down can be seen. The opportunity is then afforded for the escape into the circulation of caseous material containing tubercle bacilli. In all sites of the body where progressive tubercles are present there appears to be a chance of dissemination taking place in this way.

Another important mode of progressive dissemination is the transference of an infected focus from one site to another. Special importance here attaches to the endothelial cell, which is the commonest site to which a circulating bacillus becomes attached, both in the vascular endothelium and on the walls of the lymph sinuses. It has already been pointed out that the reception of bacilli by these cells is often followed by tissue changes which form a further barrier to the escape of the bacilli. But here attention must be called to another fate which very commonly befalls them. The cells swell up, become detached from their place in the endothelial lining and are carried off into the lymph or blood stream together with the bacilli which they contain. And a larger group of cells, or even a small tubercle growing into the lumen of a vessel, are sometimes found to share the same fate.

Mention must also be made of the spread of infection by the conveyance of bacilli-laden material along the air passages, the alimentary tract, the renal tubules, and the tubules and ducts of the mammary gland. My histological specimens afford examples of the presence of bacilli in these situations.

Taking my specimens as a whole, it has been a comparatively rare event to find the mammalian bacillus within a patent blood-vessel. I have sometimes found intravascular bacilli at early periods after subcutaneous inoculation and in the terminal stages of highly acute infections ; and after intravenous inoculations with overwhelming doses which have produced a toxic effect without lesions typical of tuberculosis I have often found bacilli in the circulation, in the later as well as in the earlier periods after infection. Some of the circulating bacilli were free, whilst others were attached to cells, much the commonest vehicle being the detached endothelial cells. The proportion of free to attached or intracellular bacilli varied very considerably in different cases. In many cases free bacilli have been in the majority in the blood ; and it is of interest to note that I have found a similar preponderance of free bacilli in tuberculous milk. In support of my histological evidence that the mammalian bacillus is unable to thrive, or even to persist, in circulating blood, I may remark that in my bacteriological work I have found pure, liquid blood serum an unfavourable culture medium ; in milk, on the other hand, I have obtained good growth, a fact which may be considered in connection with the capacity of the bacillus to lodge and multiply in the folds of the bovine milk sinuses apart from, or even in the absence of, definite tubercles of the mammary tissue.

Considering the particular types of cells wherein the bacillus may grow, I have found that endothelial and connective tissue cells and many varieties of epithelium often contain bacilli and permit of their growth *in situ* ; cell fusions and giant cells, derived from endothelium, connective tissue, and epithelium, are also favourable sites. Occasionally there is an abundant growth of intracellular bacilli which is associated with very little tissue change ; but where the growth of bacilli is most abundant the tissue is generally completely broken down and it is no longer possible to trace its cellular constituents. As to the particular regions of the body in which I have found the bacillus to grow most freely, I must first remark that, setting aside severe modes of experimental infection and experiments on highly susceptible animals, the bacillus usually produces lesions of a chronic type in which it is unable to grow in more than scanty numbers. I have rarely found abundant growth in any organ of the body except when the infection has reached an acute stage. Under these latter circumstances the lungs and the lymphatic glands are the tissues in which the bacillus grows most readily. The cow's udder is another tissue in which bacilli are often very abundant. I have also found a profuse growth of bacilli amongst the villi of the small intestine and in the lumen of the gut.

Cellular Relationships.

Proceeding to consider my observations on the reactions of the cells of the animal organism towards the mammalian tubercle bacillus, I commence with those leucocytes the protoplasm of which can, by appropriate methods, be made to stain with eosin. The most important of these cells is the one known as "finely granular" or "polymorphonuclear" ; I cannot call it "neutrophil," because by this method of staining it is oxyphil. The ordinary "coarsely granular" leucocyte is, of course, more strongly oxyphil. My method is to stain the specimens with warm carbol-fuchsin, wash with methylated spirit, then counterstain first with 5 per cent. watery eosin and afterwards with Loeffler's methylene blue. In this way I am able to give a yellowish-red tinge to the less strongly oxyphil leucocytes which are not disintegrated, and to impart a distinctly deeper red colour to the bacilli. Two advantages of the method are that bacilli contained within such leucocytes are clearly shown, and that the younger, mononuclear forms of the finely granular oxyphil leucocyte, which are common in prolonged leucocytosis and have a deeply-stained spherical nucleus quite different from that of the ordinary myelocyte, are clearly differentiated from the small lymphocyte. In some cases, where bacilli have been present in a confused mass of leucocytes, I have used the more pure yellow, Orange G, to stain the leucocytes instead of eosin. The three species of animals in which I have had the most favourable opportunities for observing different stages of infection are the bovine, the pig, and the rabbit. As will be observed from my illustrations, "finely granular" is a relative term. The size of the granules in the "polymorph" varies very considerably in histological sections of lesions, even in the same species of animal. In comparing different species the differences are found to be still greater. In the rabbit, for example, the granulation of the ordinary "polymorph" is particularly coarse. I find that the cell which is generally known as the "coarsely granular oxyphil" is rare in the lesions which I have examined and plays a quite insignificant part in relation to infection by the tubercle bacillus.

In the bovine, at early periods (1, 3, 8, and 14 days) after subcutaneous inoculation with a large dose of bacilli, the local lesion contains a dense mass of finely granular leucocytes which have surrounded the bacilli and have ingested them in large numbers. Passing outwards from the central mass of bacilli and leucocytes, bacilli are found loosely scattered about and leucocytes are plentiful but rarely contain bacilli ; they seem to be converging on the central mass and not to be attracted towards these loosely scattered bacilli. In later stages, when the bacilli are of high virulence similar leucocytes continue to invade the expanding lesion but are rapidly destroyed, though numerous fresh arrivals can be found at the periphery ; in lesions of the more chronic type, produced by less virulent bacilli, the leucocytic invasion becomes much less abundant and the leucocytes present, though still of the "finely granular" type, tend to assume a sharper granulation, and the individual granules become larger. In both types of lesion the ingestion of bacilli by leucocytes is mainly confined to the central areas where caseation is in progress. Leucocytes which have ingested or come into contact with bacilli rapidly break down ; there is no evidence that they play an important part in conveying bacilli from the site of inoculation into the blood stream.

In early lesions of the bovine lungs leucocytes, of the finely granular type, arrive rather slowly, and though a few of them are found to contain bacilli the evidence of phagocytosis is only slight. In later lesions of an acute type the influx of leucocytes is abundant, but they rapidly break down ; even up to the time of death there is generally a large influx of these cells ; definite evidence of phagocytosis is often found, but on the whole the number of leucocytes containing bacilli is small compared with the number of those which do not. The leucocytes appear to be attracted by the irritant effects of the breaking down tissue rather than by any specific stimulus exerted by the bacilli. In tubercles of an intermediate or slowly progressive type similar leucocytes are numerous, and are often particularly conspicuous at the margins of the lesions ; when bacilli are numerous a few of them are not infrequently found within leucocytes. In tubercles more definitely chronic or presenting retrogressive characters the number of leucocytes is variable, but where caseation is in progress these cells are generally found within the lesions in large numbers. At the periphery, leucocytes are frequently numerous in the interstices between the fibrous elements, and there assume a coarser granulation. After intravenous inoculation, where typical tubercles are produced the distribution of leucocytes is similar to that found after other modes of infection; where the effect of the intravenous inoculation is to set up inflammatory changes not typical of tuberculosis, leucocytes are usually distributed throughout the tissue in large numbers and some of them, generally a relatively small number, may be phagocytic.

In the bovine liver, at early periods after subcutaneous inoculation, leucocytes do not usually occur in the lesions in large numbers and are rarely found to contain bacilli. In the later stages of acute infection, broken down leucocytes are always found but the number of fresh arrivals is variable, being large in some specimens and small in others; finely granular leucocytes giving a good oxyphil stain are sometimes found to contain bacilli. In lesions of the intermediate and chronic types the number of leucocytes giving a protoplasmic stain with eosin is again variable but is usually fairly large; there is a tendency for the leucocytes to assume a coarser granulation, particularly at the margins of the lesions; evidence of phagocytosis is rare. This difference in granulation to which I refer appears due to a gradual change in those "polymorphs" which remain entangled in the tissue but do not break down; I have not found evidence of a marginal infiltration with the "coarsely granular eosinophil." In the livers of animals which have been inoculated intravenously leucocytes are attracted to places where there is tissue disintegration, but in the specimens showing diffuse infiltration of bacilli they are rarely phagocytic.

In the bovine kidney leucocytes are evidently attracted to irritant or breaking down material, but they are not closely associated with tubercle bacilli and are rarely phagocytic.

In bovine lymphatic glands the inoculation of tuberculin produces a marked increase in the number of finely granular oxyphils, even where tubercles and tubercle bacilli are absent. In early lesions, following subcutaneous inoculation of bacilli, the coarsely granular leucocytes normally present in lymphatic glands diminish in number and many finely granular leucocytes make their appearance; at first these leucocytes are not found in immediate contact with bacilli; they begin to converge upon the bacilli when the latter have produced some tissue disintegration, and when this stage has been reached the leucocytes are occasionally found to be phagocytic. In later stages of the disease, when the infection is acute, the glands rapidly give way to complete caseation and in these cases not many intact leucocytes may be found, but in diseased glands which retain some portion of their normal structure leucocytes are usually abundant and may sometimes be found ingesting bacilli at or near the periphery of caseous areas. In glands containing tubercles of the intermediate or chronic types, leucocytes are usually plentiful, both at the margins of the tubercles and in their interior, and are occasionally found containing bacilli. After intravenous inoculation leucocytes are usually numerous, but not invariably; they are often found converging upon the bacilli and are sometimes phagocytic.

In tuberculosis of the cow's udder, both in acute and in chronic infections, finely granular oxyphil leucocytes are usually abundant, and where bacilli are numerous some of them are generally found within leucocytes.

In swine, during the early periods after subcutaneous inoculation leucocytes of the finely granulated oxyphil type are attracted to deposits of bacilli more rapidly and more abundantly than in bovines, and the leucocytes are more frequently found to be phagocytic. This early and copious determination of leucocytes, even to foci where only a few bacilli have been deposited, is a striking feature of tubercle formation in the pig. Following the process to a later stage, it is found that even where the bacilli have all disappeared a large accumulation of leucocytes remains *in situ;* these cells gradually disintegrate, become surrounded with a fibrous wall, and give rise to a well marked tubercle, readily visible to the naked eye. In the calf, on the other hand, the destruction of a small number of bacilli is frequently not accompanied by an excessive aggregation of leucocytes; the damage to the tissue is less, and the process of repair often consists merely in the filling up of the lesion with a group of lymphocytes; lesions such as these would not be visible to the naked eye.

In later stages of tuberculosis in the pig leucocytes are usually very abundant in the tubercles and are more frequently phagocytic than in the calf.

In rabbits the injection of tubercle bacilli is followed by a prompt leucocytosis. The great majority of the invading cells are "polymorphs"; it must be remembered that in the rabbit this cell naturally possesses a rather coarse granulation. This leucocytic invasion is especially well marked in the local lesion produced by subcutaneous inoculation, and effectively retards the early dissemination of the great majority of bacilli inoculated. On the whole, the rabbit's leucocytes appear to be rather more active than those of the calf. Both in acute and in chronic lesions leucocytes are usually very abundant. After inoculation with dead bacilli, leucocytes usually tend to collect round the material inoculated, and where the bacilli are deposited in clumps or large groups they stimulate a very abundant aggregation of leucocytes.

In order to obtain more precise information from my specimens regarding the degree of phagocytic activity which the finely granular oxyphil leucocyte manifested towards the mammalian tubercle bacillus, I have supplemented my observations by a series of counts recording the amount of phagocytosis by these cells actually observed in my sections of representative tissues. Before making a count the whole specimen is examined, and a typical portion of it is selected as the area to be estimated. Then, with the aid of the mechanical stage, 1,000 leucocytes are counted; at the same time records are made of the number of bacilli found within the area occupied by these leucocytes, of the number of these bacilli contained within leucocytes, and of the number of leucocytes containing these bacilli. The only leucocytes counted are those the protoplasm of which takes on a stain with eosin, *i.e.*, the leucocytes which were not disintegrated when the tissue was removed from the body and fixed. "Coarsely granulars" were rarely met with; when they occurred in any appreciable numbers, a special note was made about them. The amount of phagocytosis recorded is what I term "active" phagocytosis, *i.e.*, it is based on an estimate of the proportion of intact leucocytes which actually contained bacilli at the time when the experiment was terminated.

222 counts have been made, viz., 110 in tissues of calves, 66 in rabbits, 30 in swine, and 16 in goats. In six of these the bacilli present within leucocytes could not be counted with accuracy, and in 70 the bacilli present in the area containing the thousand leucocytes were too numerous and too closely grouped together to be accurately counted.

Taking the above leucocyte counts as a whole, the most prominent feature about them is that the percentages of bacilli contained within leucocytes are very small. This is not due to any scarcity of leucocytes, because the percentages of leucocytes containing bacilli are also small, and when a bacillus finds its way into a leucocyte it is the leucocyte which is unlikely to survive rather than the bacillus; in fact, what I have constantly found in the histology of progressive tubercles is the association of dead and disintegrated leucocytes with groups of bacilli bearing evidence of multiplication *in situ.* Following the traditional custom, I have classed as actively "phagocytic" every intact leucocyte found to contain a tubercle bacillus, but it must be remembered that in reality the bacillus is killing the leucocyte, and that the leucocyte is generally not devouring the bacillus. But even on the assumption, by no means justifiable, that every bacillus within a leucocyte has been killed, the amount of phagocytosis is too small to warrant the opinion that the leucocyte is the essential or most important agent which protects the tissues against the tubercle bacillus. And the low percentage of phagocytic leucocytes cannot be accounted for by the relatively high virulence of the bacilli, since, as shown in my tables, highly virulent bacilli are quite as frequently ingested by leucocytes as slightly virulent bacilli.

It is useful to compare these leucocytic counts with my observations, recorded in earlier paragraphs, on the presence of leucocytes in local lesions produced by subcutaneous inoculation. In the central area of these lesions the inoculated material is infiltrated with a dense mass of leucocytes which are stuffed with bacilli. Confining one's attention to such a site as this, the leucocytes would, in ordinary phraseology, be described as intensely phagocytic. But on passing outwards from the central point of the inoculated material, a different impression is created. Bacilli are freely distributed, and there are plenty of leucocytes; but relatively few of the leucocytes contain bacilli. And, again, on passing from the local lesion to deposits of bacilli in distant organs of the same animal, the amount of phagocytosis is found to be still smaller. Therefore, the abundant phagocytosis found in the mixed mass of leucocytes and bacilli is no indication of the amount of phagocytosis taking place in other parts of the body. In view of these differences in the amount of leucocytic phagocytosis found to take place in different parts of the same body at the same time, it is difficult to understand how the phagocytosis obtained *in vitro* with leucocytes and tubercle bacilli can afford reliable information as to the phagocytic activity of the leucocytes in a particular patient at a particular time.

As theories based upon the phagocytic activity attributed to particular cells are often supposed to be supported by the authority of Metchnikoff, it is only right to point out that Metchnikoff ascribes phagocytic activity towards the tubercle bacillus to the macrophage rather than to the microphage, though he lays stress on the "afflux" of microphages. "Even in the examples where it is, at first, principally the macrophages which destroy the micro-oragnisms (as in the case of the resistance of the animal organism against the tubercle bacillus), there is also a great afflux of microphages." (Binnie's Translation of Metchnikoff's *Immunity in Infective Diseases*, p. 177.) My observations are consistent with this statement, both as regards the afflux of microphages and as regards the fact that tubercle bacilli are often present within, and often perish within, cells comprised under the conveniently elastic category of "macrophages."

Attention may also be called to the following points in connection with my observations on phagocytosis:—

(1.) In the tissues of calves experimentally inoculated after previous vaccination the amount of leucocytic phagocytosis is much smaller than in the control animals, inoculated with the test dose without previous vaccination.

(2.) In terminal infections after subcutaneous inoculation with bacilli of high virulence, the percentage of leucocytes containing bacilli is much higher than in infections after intravenous inoculations, most of which were fatal and were caused by viruses of lower virulence.

(3.) The leucocytes of swine are found to ingest tubercle bacilli more frequently than the bovine's leucocytes, whilst the leucocytes of the rabbit are less active than the former but more active than the latter. Thus, taking comparable cases, where precise estimates were possible, amongst bovines in 25 areas each containing 1,000 leucocytes a total of 2,188 bacilli was counted, the number of bacilli in leucocytes being 36, and the percentage of bacilli in leucocytes 1·6; in 25 similar areas in swine tissues a total of 5,301 bacilli was counted, the number of bacilli in leucocytes being 323, and the percentage of bacilli in leucocytes 6·1. In rabbits, in 37 areas each containing 1,000 leucocytes a total of 8,133 bacilli was counted, the number of bacilli in leucocytes being 275, and the percentage of bacilli in leucocytes 3·4.

My observations on the small lymphocyte are in accordance with the generally accepted view that this cell is not phagocytic and that it is not directly attracted to the tubercle bacillus. Occasionally I have found such cells in contact with bacilli, but such association has appeared to me to be clearly accidental. I have observed, however, that with the tissue changes set up by the tubercle bacillus these cells are constantly associated, and are present in numbers which vary very greatly under different conditions.

In local lesions at an early period after subcutaneous inoculation the great majority of the infiltrating cells are finely granular oxyphil leucocytes; small lymphocytes are also present but are relatively scanty. During the second week a marked increase is noted in the number of lymphocytes, which form aggregations at the periphery of the lesions. During the second week and subsequently, the abundance and distribution of the small lymphocytes is determined by the severity of the lesion. When the lesion is rapidly progressive the lymphocytes are confined to the periphery, forming a loosely scattered zone which recedes as the area of necrosis advances; but when the lesion is not advancing rapidly the lymphocytes form a much denser peripheral zone and tend to advance into the interior.

In early disseminated lesions I cannot lay down any definite rule regarding the infiltration of small lymphocytes and the relations of these cells to the leucocytes. There is not, for example, a particular stage in the process during which the infiltrating cells are lymphocytes, another period, earlier or later, when they are leucocytes, and so forth. In most early lesions some lymphocytes are to be found, and the relative abundance of these cells appears to be determined in each particular site by the degree of local irritation which is present. Lymphocytes are present where the amount of tissue damage is slight, but when the tissue is rapidly breaking down they are only found at the outskirts of the lesion.

Similarly, in later stages of the disease, the distribution of small lymphocytes varies according to the type of lesion produced. In acute infections these cells are loosely scattered and do not form a compact peripheral zone, though they are more numerous at the periphery than in the interior of the lesions. In milder types of infection the peripheral zone of lymphocytes is often very dense, and in the type of mild, obviously retrogressive lesion which has not given rise to fibrous tissue formation the site may be almost completely filled up with lymphocytes.

In examining my specimens with a view to ascertaining the part played by the endothelial cell, I find that, owing to the variety of forms which it may assume, this cell is more difficult to trace than the oxyphil leucocyte or the small lymphocyte. This difficulty is increased by the fact that some of the forms assumed by endothelial cells are identical with those assumed by cells not derived from endothelium; hence it is often quite impossible, in dealing with the confused mass of cells usually encountered in tubercular lesions, to find any morphological criterion by which all the cells of endothelial origin, except those lining definite spaces or channels, can be identified. The simplest plan is to commence with facts about which there can be no dispute. In tissues infected by the tubercle bacillus, have found the following changes, in addition to merely degenerative change, in cells which are unmistakably endothelial:—(1) swelling, (2) diffuse staining of nucleus, (3) increase of nuclei by direct division, (4) proliferation. The occurrence of each of these conditions will be considered separately, and then (5) the relations of the endothelial cell, in its various phases, to the tubercle bacillus will be dealt with.

In early infections it is constantly found that the endothelial cells of the affected area become swollen. This change must be attributed to some diffusible irritant associated with the presence of bacilli; it is not confined to the endothelial cells with which bacilli are actually in contact.

Swollen endothelial cells often become detached and pass into the fluid contained within the endothelial lining. They then present the appearance of what are sometimes called "macrophages."

It is a marked characteristic of the tubercle bacillus to become attached to, or enter within, an endothelial cell; hence these cells, becoming detached and circulating in the lymph and blood-streams, form an important vehicle for the conveyance of tubercle bacilli.

In the process of tubercle formation, swollen endothelial cells often lose their continuity with an endothelial lining and are at the same time cut off from access to the lymph or blood-stream. They then present the appearance of what are sometimes termed "epithelioid" cells.

Associated with the swelling up of those endothelial cells which are in direct contact with bacilli, there are changes in the nucleus. The nucleus generally stains more darkly than the normal, and the material taking this stain often stains diffusely and tends to escape into the surrounding protoplasm. These changes form noticeable features in sections of tissue containing very early lesions; they present the appearance of minute foci wherein the excess of nuclear stain has not been properly washed out, and hence can be readily discovered in searching under a low power a piece of tissue which is otherwise normal.

Whilst these nuclear changes are going on there is also to be observed a dissolution of continuity in the protoplasmic outline and a tendency for the endothelial protoplasm to fuse with the protoplasm of contiguous parenchymatous or other cells. It is of particular importance to note, in relation to the part played by the endothelial cell in tubercle formation, that at this stage the endothelial cell begins to lose its individuality.

The nuclei of these altered endothelial cells exhibit a very strongly marked tendency to multiply by direct division. First the nucleus exhibits an irregularity of outline, a bulging or an indentation at one side or the other; then portions of nuclei are seen to be partially split off, and in the next stage a bunch of nuclei, each with a distinct outline, is found. This nuclear multiplication, when abundant, contributes largely, though not exclusively, to the nuclei of what are termed "giant cells."

In the early stages after infection I have found, in addition to the changes above noted, that some of the endothelial cells exhibit regular mitotic figures and divide in the normal fashion. But when definite tubercles are developing, the endothelial cells included within these lesions do not exhibit nuclear mitoses. The cells at the periphery of the lesions usually contribute, by direct division, to the formation of multinucleate cell masses; but, provided that they retain their protoplasmic outline, *i.e.*, remain individual cells, they may divide by mitosis and, preserving their character as endothelium, contribute to the formation of new capillary channels. The endothelial cells immediately external to the periphery of the tubercle may also assist in the process of new capillary formation; but in acute lesions they fail to proliferate, and in retrogressive lesions they give way to fibroid change.

Endothelial cells, I have observed, readily ingest tubercle bacilli, and in that sense are highly phagocytic. But the cells containing bacilli lose their normal morphological structure, and it does not appear likely that they are capable of returning to the normal. In these altered endothelial cells the bacilli may perish, and frequently do; but there is also abundant evidence that the bacillus may both retain its vitality and grow in these situations. I also find that it is a marked characteristic of the mammalian tubercle bacillus to become attached to, or to pass within, an endothelial cell, and that such cells, becoming detached and circulating in the lymph and blood streams, form an important vehicle for the conveyance of the bacilli.

I have found that cells with the staining characteristics of Unna's plasma cells are almost always present in tissues infected by the tubercle bacillus. Their presence must be due to some pathological change associated with the infection, since they do not occur, or only occur exceptionally and in scanty numbers, in normal specimens taken from corresponding tissues, such as the lung, liver, kidney, or mammary gland. Owing to the difficulty of the problem regarding the origin and nature of these cells and their relation to certain cells found in lymphatic glands and in the blood, I find that the simplest plan is to commence with the fact that certain cells give a characteristic staining reaction, to call these cells "plasma" cells, and to note their relations to infected areas. And as cells with similar staining properties are normally present in lymphatic glands, it is an advantage to consider first lesions not involving lymphoid tissue. The differential stain which I employ is Pappenheim's combination of pyronin and methyl green. The protoplasm of the plasma cell takes up a definite red stain, in contradistinction to the protoplasm of the other cells present, which either do not retain the pyronin stain at all or only show a slight trace of it. Morphologically the plasma cell presents great diversity. Sometimes it is indistinguishable from an ordinary large lymphocyte; it is often compressed, and rectangular in outline; it not unfrequently has tapering extremities; and it is very often characterised by the eccentric position of its nucleus.

During the first week after infection plasma cells are sometimes found in relation to tubercular lesions but are relatively scanty. During the second week they form a much more conspicuous element and tend to aggregate at the periphery of the tubercles.

In later stages of infection, when the disease is of an acute type, plasma cells, though generally demonstrable, rapidly break down; they are more readily demonstrable at the periphery of lesions but do not form a well defined zone. In less severe and in chronic infections the plasma cells generally form a conspicuous and abundant element at the periphery of the tubercles. I have also observed that at a later period if, owing to the mild or chronic character of the infection, these cells retain their vitality, they again become narrow and elongated and resolve themselves into fibro-blasts.

I find that plasma cells are not phagocytic; they perish when brought into proximity to tubercle bacilli. They do not form giant cells, and their nuclei rarely multiply by direct division.

Examining tissues showing a progressing infiltration of cells derived from the blood, I usually find that the blood-vessels through which the section passes contain such cells in excess of the normal. This is the case, for example, in leucocytic infiltrations, unless the tissues happen to be removed at a time when the process of immigration is not active. But in examining sections showing various stages in the appearance of plasma cells, it seems to me a significant fact that at no stage is it found that cells which could possibly be mistaken for plasma cells are at all numerous in the blood-channels. The only cells not distinguishable with certainty from plasma cells are the large lymphocytes. These latter cells are sometimes to be found in vessels permeating tissues where plasma cells are demonstrable, but the number of such lymphocytes is so small, and the association of their presence with the occurrence of plasma cells in the tissues is so inconstant, that it seems to me extremely unlikely that the latter cells have been conveyed by the blood-stream. I have observed another very strong piece of evidence in favour of the local origin of the plasma cell. Whenever these cells are at all numerous, especially in the case of developing lesions, some of them, and frequently a considerable number of them, show nuclear mitoses. The very early appearance of plasma cells in the connective tissue spaces in proximity to a newly formed lesion is also suggestive that these situations are the sites of their origin.

It therefore appears from my investigations that plasma cells are not derived from the blood, but originate from cells normally present in the connective tissues, particularly at the periphery of vessels and bronchioles and in the septa between the parenchymatous cells of various organs. The diffusible irritant caused by the action of the tubercle bacillus upon the tissues stimulates these cells and sets up changes in them whereby they respond to the differential staining reaction of plasma cells. The cells so stimulated expand, change from an elongated or irregular to a more or less spherical form, very frequently multiply by indirect division, and migrate towards the periphery of the tubercular focus.

In association with the lesions found in infected lymphatic glands, cells are found which are in every respect identical with the plasma cells found in tubercles occurring in other organs; and as regards the variations in the numbers, morphology, and relationships of these cells in accordance with the severity of the lesions, I find that lymphatic glands show a correspondence with other organs. In the lymph glands, as elsewhere, I have found evidence that the cells in question are produced *in situ*, and have particularly noted that many of them show mitotic figures when present in association with developing lesions. But owing to the fact that the large lymphocyte normally found in the lymphatic gland takes the characteristic staining reaction of the plasma cell, it is impossible in such tissue to decide whether any particular cells resembling large lymphocytes are or are not cells capable of developing the variations in morphology which are typical of plasma cells.

In endeavouring to follow the fate of epithelial cells I have found the same difficulty as with endothelial cells. In particular tissues where pathological change has set in it has often been impossible to say what cells were epithelial orginally and what were not. But the examination and comparison of a large number of specimens, including early lesions, enables me to say something about what happens to the epithelial cell.

In early infections of the lungs the first change noted is a localised swelling up of the pulmonary epithelium, and when the infection is severe this change is rapidly followed by desquamation. The epithelial cells sometimes show mitotic figures, but much more frequently their nuclei divide by direct division. In the stage preceding caseation their protoplasm loses its outline and fuses with the protoplasm of adjacent cells, endothelial or epithelial. There is then found a multinucleate mass of protoplasm in which it is no longer possible to identify the epithelial element. But at the periphery of such an area swollen, distorted, and faintly outlined epithelial cells often survive and are then usually termed "epithelioid." In highly acute infections, however, the swelling up, desquamation, and disintegration of the epithelial cells follow one another in such rapid succession that the general appearance is that of diffuse progressive necrosis without any arrangement of cells or cell masses into distinct tubercles. In later lesions of a definitely retrogressive type the epithelial element has completely disappeared; but in the circumscribed, progerssive tubercle desquamated epithelial cells are generally conspicuous in the alveoli adjacent to the lesion. The pulmonary epithelial cell readily ingests tubercle bacilli and appears to be a site favourable for their multiplication.

In specimens of liver examined after very recent infection tubercle bacilli are occasionally found in parenchymatous cells histologically normal. But it is the general rule that the presence of a tubercle bacillus almost immediately damages the protoplasm of a liver cell and causes distortion and irregular staining of the nucleus. The cells then lose the continuity of their protoplasmic outline and their nuclei begin to segment by direct division. There is then found a confusion of nuclei in protoplasmic material, and it is often impossible to say how much of this material originates from the liver cells and how much from the surrounding endothelium. It is however clear, in following up the development of early lesions, that the liver cells contribute a considerable share both to the protoplasm and to the newly formed nuclei of the resultant tubercle.

Both in the kidney and in the mammary gland the epithelial cells rapidly degenerate when tubercle formation commences; they contribute to the caseous element, but their nuclei rarely show signs of multiplication.

In addition to the wandering cells already alluded to, I must refer to my observations on the large lymphocyte or hyaline cell which is found, normally, both in lympathic tissue and in the circulation.

In specimens stained by Pappenheim's method the protoplasm of the large lymphocyte or hyaline cell takes up the pyronin stain. In the lymphatic glands these cells generally take a strong protoplasmic stain; when found within sections of blood-vessels they stain less deeply. In the lymphatic glands these cells rapidly disappear from sites where bacilli are present; they are not phagocytic and cannot survive in close contact with bacilli; bacilli are only found in contact with them or within them when the cells are breaking down. When found in the blood-vessels these cells do not contain bacilli. Amongst the wandering cells which make their appearance in the tissues when tubercles are in process of development some of these large lymphocytes which have escaped from the blood-vessels may occur; but only a small number of these cells are found in the circulation, and there is no evidence that many of the cells derived from this source have emigrated into the tissues surrounding tubercles. When present, their staining reaction does not distinguish them with certainty from the plasma cells, which are of local origin; but as regards their relations to tubercles and tubercle bacilli, what has been said of plasma cells applies to all cells reacting to Pappenheim's stain, and therefore includes these cells. They do not play an active part in tubercle formation and do not ingest bacilli. These lymphocytes are to be distinguished from the large mononuclear wandering cells which do not take up the pyronin stain by Pappenheim's method, and are actively phagocytic. The latter cells are of endothelial origin.

I have also paid attention to the relations of lesions produced by the tubercle bacillus to compact fibrous tissue, loose connective tissue, and elastic tissue.

Compact fibrous tissue is not readily attacked by tubercle bacilli, but forms an important barrier against dissemination. This is well illustrated by the fibrous septa of the udder, in mammary tuberculosis. Similarly the newly developed fibrous tissue, of the adult type, which is found at the margin of retrogressive tubercles, affords strong evidence that the expansion of the lesion has been arrested. But in acute infections the natural barriers of pre-existing fibrous tissue are gradually worn away and break down.

Loose connective tissue exhibits much more active change. At a very early stage in the infection the connective tissue corpuscles swell up and show signs of nuclear division by mitosis. New corpuscles are formed, but when this stage has been reached it is generally impossible to distinguish these cells with certainty from cells of endothelial origin, as the latter are also undergoing active change. When these new connective tissue corpuscles degenerate probably many of them share the same fate as the endothelial cells, *i.e.* their nuclei divide by amitosis without corresponding division of the protoplasm; multinucleate cells are thus formed, and eventually giant cells. When the infection arrives at a retrogressive stage the young connective tissue corpuscles which have survived without degenerating tend to form fibrous tissue of the adult type.

The swollen connective tissue corpuscle very commonly contains bacilli, and frequently intracellular bacilli are found in large numbers. In this sense it may be said to be phagocytic; but it appears rather that the bacilli often find in this type of cell a favourable site in which to multiply, and may succeed in multiplying without causing the cell to lose the continuity of its protoplasmic outline.

In lesions of an acute type the normal elastic tissue, as demonstrated by Unna's orcein method, gradually becomes eroded and disappears, but its disappearance is not so rapid as that of the cellular constituents. In the lungs, for example, it is often possible to trace the outlines of alveoli in consolidated and degenerating patches by means of the threads of elastic tissue which survive. In less acute lesions there is also a gradual disappearance of the elastic elements. Neither in acute nor in chronic lesions have I found any signs of new formation of elastic fibres. It is noticeable, more particularly in the chronic, caseating lesions, that more elastic fibres have survived at the periphery than at the centre; and in an expanding lesion, *e.g.* in the lungs, it is natural to expect that the compressed or collapsed tissue at the periphery should show these elements in relatively large amount. In the tubercular outgrowths which develop on the surface of the pulmonary pleura the absence of elastic elements in the lesions is in marked contrast to the elastic lining of the pleura and the elastic network in the subjacent lung tissue.

Another important aspect of the lesions which I have studied is the relation of the infective processes to vascular degeneration and growth. I find that the vascular channels originally present tend to degenerate when they become involved in a tubercular focus. This degeneration often proceeds rapidly, the consequence being that tubercles are very frequently found in which all vascular channels have entirely disappeared. But my observations do not justify the broader generalisation that the tubercle is essentially a non-vascular structure. Within both slowly progressive and rapidly progressive lesions patent vessels, including capillaries, are often found; and capillaries are often found where the walls are just on the point of giving way and allowing access to the bacilli contained in the surrounding tissue. The relatively late survival and subsequent disruption of vascular channels in infected areas are features which favour the dissemination of infective material.

The outer coats of the larger vessels are often found to be greatly thickened, and another change is occasionally noted, the development of tubercles in their endothelial lining. These tubercles break down, as caseation advances, and discharge their contents, including bacilli, into the circulation.

New capillaries are formed, or tend to be formed, from pre-existing endothelial cells or young connective tissue corpuscles, wherever there is proliferation of these elements in the normal way. With the exception of the two extremes, the completely quiescent lesion where there is no tissue activity and the acute lesion where all the cells are breaking down, there is the possibility that the infected areas may show some attempt at new capillary formation. In many of these lesions, however, the cells capable of forming endothelium do not show a normal activity, but tend to divide amitotically, and generally without concomitant division of the protoplasm; the cases where the infected areas contain cells of the type which proliferates into normal daughter cells are less frequent but by no means uncommon, and under these latter circumstances columns of cells forming rudimentary capillaries are seen. Tubercles formed in loose connective tissue or as outgrowths on the surface of the lungs or liver provide good examples of the free development of new capillaries; the development of capillaries at the periphery of compact tubercles is more restricted and less perfect.

In studying cell degenerations I have observed that in sections stained for fat, there may be found a very conspicuous deposit of fat globules amongst the cells bordering on caseous areas, or in cells at the commencement of caseous change. Numerous fat globules are also found lying free in the caseous material.

Another conspicuous feature of many of my specimens is the presence of multinucleate cell masses. I have observed that cells of all kinds may contribute to the protoplasmic matrix of a multinucleate cell mass, and their nuclei may be found embedded in this material. But the nuclei which augment the nuclear aggregation by dividing amitotically are derived from one or more of a smaller number of cells. The nuclei of endothelial and connective tissue cells and of epithelial cells, particularly epithelial cells of the lung or liver, are capable of dividing in this way; but I have not observed this process, in association with multinucleate cell formation, in plasma cells, large lymphocytes, small lymphocytes, or oxyphil leucocytes. The so-called "giant" cell is merely the maximum expression or the most striking example of this tendency to form a multinucleate cell mass. The general or fundamental process is a tendency to cell fusion, with a concomitant loss of cell individuality. This process is always in operation in tubercle formation; the formation of the giant cell is merely an incident, which may be absent.

For the purpose of studying fibrin-formation I have stained my specimens by Kochel's method, and in order to observe the relations of the bacilli to the fibrinous deposits I have stained the sections for bacilli before applying the fibrin stain. I have found that deposits of fibrin are associated with acute infections. In chronic lesions of a mild or retrogressive type some of the degenerative products may not be completely decolourised when a differential stain for fibrin is applied, but these are distinguishable from fibrin filaments.

In calves subcutaneously inoculated with a large dose of virulent bacilli the lungs do not show fibrin in very early stages of the infection, but a few small deposits can be found in 14 days (Calf 288). In the later stages of acute infection in the bovine after subcutaneous inoculation, deposits of fibrin are always formed, and in specimens illustrating the terminal stage of the disease both old and recent deposits can generally be found in large amount. The fibrin occurs in the consolidated areas and sometimes in the blood-vessels. In the case of foci where necrotic change has commenced the fibrin is more sharply defined at the periphery than in the centre. The distribution of the fibrin is highly irregular; individual alveoli and groups of alveoli are often filled with this deposit whilst the adjacent tissue contains none. This irregularity appears to be a characteristic feature of the disease. Tubercle bacilli are always found in association with the fibrinous deposits, but on the other hand the presence of bacilli is not always associated with fibrin. In the acute infections produced by subcutaneous injection of 50 mg. the duration of the experiments varied from about three weeks to twelve weeks, but no corresponding difference is observable in the amount of fibrinous deposit found in the lungs, or in its character. In all cases both old and recent deposits can generally be found. In Calf 787, for example, which died at an unusually late date (81 days after inoculation) many of the fibrin filaments are delicate and evidently of recent origin. It seems clear, therefore, that fibrin formation is not a single event but occurs piecemeal at different periods throughout the later stages of acute infection. In acute lesions of the lungs following subcutaneous inoculation with tissue emulsions, where the dose of bacilli was much smaller than in the culture inoculations, fibrin formation takes place in the same way as where large doses have been inoculated. In many instances strains of bacilli, found to be of low virulence when inoculated subcutaneously, have been found, when introduced intravenously, to produce death in calves during the third or fourth week. In these cases, though the lungs contain very large numbers of bacilli, little or no fibrin is present as a rule, but exceptions have been found.

In the case of monkeys, which are more highly susceptible than bovines, where the lungs are severely affected fibrin is frequently found, but not invariably. It is absent in some of the very severe cases, and it appears as though a very high toxicity of the bacillus may be accompanied by an absence of fibrin formation.

In lymphatic glands infected with virulent bacilli fibrin very soon appears. It may be found at the end of the first week, and at later periods is almost constantly met with in association with lesions of the acute type where bacilli are at all numerous. The deposits of fibrin are localised and are confined to areas where bacilli are present. When a gland contains early disseminated foci the fibrin stain picks out these lesions very sharply. As the lesions advance, successive deposits of fibrin appear at the peripheral areas and the earlier deposits become blurred and stain less deeply.

In the liver acute infection is usually associated with fibrin formation. The fibrin makes its first appearance at an early stage and may be found in very small lesions, but is not usually formed until the cells show definite signs of breaking down.

In the kidneys fibrin formation occurs much less frequently. The lesions in these organs generally commence as an infiltration of the intertubular tissue, and at this stage deposits of fibrin are rarely found. When larger and definitely caseous tubercles are present, occasionally, but not very frequently, there is a scanty deposit of fibrin at their periphery.

In the cow's udder, when acutely infected, fibrin formation is abundant and commences at an early stage. It spreads along the intertubular tissue in association with the advance of the bacilli.

The following is a summary of my observations on caseation and calcification.

When cells are in contact with tubercle bacilli or contain them, they usually break down. Some cells, notably finely granular oxyphil leucocytes, invariably break down when they contain tubercle bacilli. Breaking down cells, under these conditions, appear to liberate material which attracts a fresh supply of leucocytes; and, unless the lesion is slight and on the road to healing, the new arrivals speedily disintegrate. At this stage there is caseation.

It seems possible that the ferments released by the disintegrating leucocytes may, when associated with the toxins released from disintegrated tubercle bacilli, assist in the formation of caseous material. The living tubercle bacillus alone does not necessarily produce caseation. In the tissues of some resistant animals, such as the rat, tubercle bacilli may be present in enormous numbers, many of them intracellular, without causing any caseous change. And in animals such as the calf, where caseation usually follows deposits of numerous bacilli, this condition may, under some circumstances, be absent. After intravenous inoculation, terminating fatally, with some strains of human bacilli, the lungs may be swarming with bacilli without showing caseous degeneration. And sometimes in bovines inoculated with bovine bacilli, it is found that the organisms have multiplied very abundantly in areas showing no caseous change. In non-caseous tissues containing numerous bacilli leucocytes are often scanty; but where caseation is present leucocytes are usually found, and there is generally a mass of broken up nuclear material which is derived from these cells. And local lesions, where caseation takes place very rapidly, contain in the initial stages enormous numbers of leucocytes.

Caseation may follow the inoculation of dead bacilli. In the lungs of Rabbit 488, for example, there are typical caseous tubercles containing deposits of the inoculated material (dead culture) in the centre Here again the lesions contain very large numbers of oxyphil leucocytes and the remains of these cells. An interesting feature of this specimen is that the disintegrating bacilli are surrounded with material which stains strongly with eosin but is not acid-fast. At the periphery of clumps of bacilli this material presents a radial arrangement of club-shaped deposits strongly suggestive of a mycelium.

The occurrence of calcification is not necessarily a sign that a retrogressive stage has been reached, either in the disease generally or in the particular lesions concerned. In the bovine, calcification of the central parts of caseous tubercles sets in very rapidly and is often found where the disease is actively progressive. It is also worth noting that bacilli may sometimes be found in the midst of calcareous deposits, and occasionally they are more numerous in the calcareous centre of a tubercle than in the surrounding caseous zone.

Putting together briefly the results of my observations on the processes of tubercle formation in the tissues which I have examined, I may state them as follows:—

The first step in tubercle formation is the penetration of an endothelial surface by a tubercle bacillus. Changes then occur in the endothelial cell. Its protoplasm becomes swollen; the nucleus stains more deeply, allows some escape of chromatin into the protoplasm, and shows a tendency to multiply by direct division. These changes vary in amount, according to the degree of local irritation which the individual cell experiences. The fixed tissue cells upon which the endothelial cells rest are also affected; their protoplasm fuses with that of the adjacent endothelial cells and is occupied by bacilli, whilst their nuclei assume irregular shapes and often multiply by direct division. There is then a progressive loss of cellular individuality. The focus which the bacilli have penetrated becomes a protoplasmic matrix containing nuclei; it no longer behaves like a collection of discrete cells, and it never subsequently returns to that condition. The nuclei may, for a time, continue to multiply by direct division, but the protoplasmic matrix never becomes differentiated into a structure composed of individual cells; its usual fate is to caseate.

These are the changes which have appeared to me typical of tubercle formation. They are accompanied by accessory changes due to irritant or inflammatory effects. The first and most noticeable of these secondary effects is the attraction of finely granular oxyphil leucocytes to the site of injury. These cells invariably break down when they come into close proximity to tubercle bacilli, and their disintegrated remains form a large constituent of the caseous element in the tubercle. How far they perform a useful function is less clear. It is at all events certain in the tissues I have examined that the degree of resistance offered by the animal organism is in no way proportionate to the amount of leucocytosis. The leucocytes are attracted just as copiously to the acutely progressive lesions as they are to lesions of a less severe character. There is, however, a difference in the result. The leucocytes which make their way into a chronic lesion preserve their integrity for a much longer period than those which have been attracted to an acute lesion.

The small lymphocyte is also constantly found at the margin of lesions in all stages of infection. This cell is never able to retain its vitality in situations where active disease is going on, but it accumulates in large numbers at the periphery of the more chronic tubercles, and generally fills up the interior of lesions which have become completely quiescent, no longer contain bacilli, and have not advanced to caseation.

Then there is a further series of accessory changes which appear due to the local irritant action of the tubercle upon the surrounding tissue, and consist of the reactions of this tissue in the directions of inflammation and repair. The characteristic features of the inflammatory stage are the swelling up of the endothelial and connective tissue elements, and a stimulus to the local production of large, mononuclear wandering cells (plasma cells), which arise from cells normally present in the adjacent connective tissue, assume a spherical form, multiply by indirect division, and migrate towards the inflamed area. The stage of repair, or attempted repair, is characterised by the formation of new endothelium and new fibrous tissue. The new endothelium, arising from pre-existing endothelial and connective tissue elements, grows partly in the form of capillary tubes and partly as a flattened layer which forms a lining to the spaces arising in the newly formed tissue. The new fibrous tissue is produced from the plasma cells, endothelial cells, and connective tissue cells, and forms a zone at the periphery of the inflamed area.

The above description appears to me to contain all the essential features of tubercle formation. Without introducing the terms "epithelioid cells" and "giant cell," it contains the histological facts which are commonly designated by these terms. It is often convenient, for merely descriptive purposes, to use the term "giant cell" as a brief designation of a continuous mass of protoplasm containing many nuclei; and I see no harm in using "epithelioid" as a convenient designation for any group of cells which bears some resemblance to epithelium in that it is a continuous layer composed of cells with a large amount of protoplasm, and each with a more or less spherical nucleus. The danger in the use of these terms is, it appears to me, that it often implies acceptance of erroneous theories. The "giant cell," I find, has neither independence nor any other characteristic of individuality, but is merely a degeneration product. It seems particularly absurd to call it a "macrophage," since it is perfectly helpless, absolutely non-motile, and forms one of the commonest sites in which the tubercle bacillus survives and grows. It is, I think, equally erroneous to assume that the tubercle bacillus is endowed with the capacity of producing, in some mysterious way, a new sort of cell with peculiar "epithelioid" characteristics. I find no mystery about those cells in a tubercle which "look like epithelium." Some of them simply are original epithelium with damaged and, in consequence, badly staining protoplasm. In other cases they are new formations; they then consist of endothelium which has grown as a covering over the walls of newly formed tissue spaces.

The pathogenic peculiarities of the mammalian tubercle bacilli which I have investigated consist in (1) their slowly toxic action upon cell protoplasm, and (2) their irritant action upon cell nuclei. (1) leads to caseous degeneration, a process for which the products of disintegrated bacilli are probably to a large extent responsible; (2) leads to the splitting up of the nuclei by direct division, a process which may continue as long as the nuclei are able to derive any nutriment from the damaged protoplasm.

B.—The General Results of the Infection of Fowls by Mammalian Tubercle Bacilli.

The following is a brief outline of my observations on this subject; the details have been recorded in previous pages of the present volume.

Dissemination of Bacilli.

After Feeding.

After this mode of infection no evidence of dissemination has been found in the specimens examined.

After Subcutaneous or Intraperitoneal Inoculation.

As a rule no evidence of dissemination is found. In the case of Fowl 186, inoculated intraperitoneally, I have found numerous bacilli in a cervical gland.

After Intravenous Inoculation.

In some cases the bacilli have completely disappeared, but more frequently they can be found in one or more of the organs. The following table illustrates the amount of dissemination found in the specimens I have examined:—

DISSEMINATION OF MAMMALIAN BACILLI IN THE FOWL AFTER INTRAVENOUS INJECTION.

Duration of Experiment in Days.	Virus.	Dose.	Number of Animal.	Presence or Absence of Bacilli in Various Organs.			
				Lungs.	Liver.	Kidneys.	Spleen.
11	B XXVI.	10 mg.	458	Many large groups	Many small deposits.	Rare	Scanty.
12	H 53. D.H.	50 mg.	21	Many small groups	Numerous small groups.	A few small groups.	Numerous small groups.
14	H 53. D.H.	10 mg.	19	Many small groups	Numerous small groups.	A few small groups.	Numerous small groups.
19	B XXVI.	6 mg.	338	Numerous	Moderate numbers; many fragmentary.	Rare	Many small deposits.
20	B IV.	10 mg.	420	Generally distributed in moderate numbers.	Scanty	A few	Scanty.
21	B IV.	10 mg.	414	Many minute foci containing numerous bacilli.	Many minute foci, each containing several bacilli.	Absent	Fairly numerous.
27	B XXVI.	10 mg.	412	Generally distributed in moderate numbers.	Many small foci, each containing a few bacilli.	Very rare	Many caseous foci, each containing a few bacilli.
30	H 107 H.H.	10 mg.	143	Many small foci, each containing several bacilli.	Many small deposits.	A few	Many small deposits.
35	B IX.	20 mg. of dead bacilli.	492	Many caseous patches containing numerous bacilli.	Very rare	Very rare	Very rare.
35	E I.	10 mg. of dead bacilli.	490	Many fragments	Very rare	Absent	Very rare.
37	E I.	20 mg.	234	Fairly numerous; fragments.	Rather scanty; fragments.	Rare	Fairly numerous; fragments.
40	H 115. N.G.	100 mg. of dead bacilli.	133	Many small deposits.	Scanty	Absent	Distributed throughout in scanty numbers.
51	H 81. P.W.	40 mg.	131	Absent	Absent	Absent	Absent.
53	B XXVI.	10 mg.	82	Many large groups	Rare	A few small groups.	Scanty; many fragments.
59	E IV.	20 mg.	136	Fairly numerous	Many small groups.	Absent	Numerous.
90	H 68. R.B.	10 mg.	15	Absent	Absent	Absent	A few fragments.
90	H 68. R.B.	50 mg.	16	Absent	Absent	Absent	A few fragments.
98	E IV.	1 mg.	134	A few groups; fragments.	Many small groups; fragments.	Rare	A few fragments.
102	B XXVI.	1 mg.	80	Many large groups	Rare	Absent	Scanty; many fragments.
103	P XLI.	15 mg.	128	Absent	Absent	Absent	Absent.
115	E III.	1 mg.	154	Absent	Fairly numerous	Absent	Fairly numerous.
121	H 49. T.C.	50 mg.	37	2 T.B.	Absent	Absent	Absent.

Destruction, Arrest, and Multiplication of Bacilli.

The above table indicates that, after intravenous inoculation, there is an initial distribution of bacilli in each of the four organs examined; but in the majority of cases the bacilli have not been able to survive for a very long period, and, when present, show signs of disintegration. In the lungs of Fowl 80 and in the liver and spleen of Fowl 154 the bacilli have survived for exceptionally long periods; both these animals were inoculated with viruses of high virulence for calves and other mammals (B XXVI and E III). Lengthy exposure to the high temperature of the fowl's body is, therefore, not invariably fatal to the bacillus.

When present in very small numbers the bacilli do not produce any noticeable tissue change, but they are generally in contact with, or within, some of the fixed tissue cells; they are not found in the circulation. When they occur in small groups, the bacilli usually cause a small localised patch of partial necrosis; if present in larger numbers, the necrotic area is more extensive and is generally surrounded by a well marked fibrous zone. Under all conditions, therefore, there is a marked tendency for the bacilli to be permanently detained in the site where they have been deposited.

When not actually broken up into fragments, the bacilli often show no deviation from the serum-grown type and appear to be unaltered survivals of the material inoculated. But in some cases they show definite signs of proliferation, *e.g.*, in the lungs of Fowls 80, 82, and 458, and in the livers and spleens of Fowls 19 and 21.

The inoculation of dead bacilli produces, in some cases, lesions similar to those caused by living bacilli.

Reaction of the Tissue Cells.

Oxyphil Leucocytes and Small Lymphocytes.

In Fowl 21, which died 12 days after intravenous inoculation with 50 mg., the lungs contain many deposits of bacilli which have produced patches of slight necrosis; in these areas oxyphil leucocytes are rarely met with, but throughout the rest of the tissue they are largely in excess of the normal; small lymphocytes are also generally distributed in large numbers, except in the effected areas. In the liver, which contains numerous small groups of bacilli, there is a general infiltration of lymphocytes and leucocytes; this infiltration is particularly dense at the periphery of blood-vessels; the cells do not appear to be specially attracted to the immediate vicinity of the bacilli, but in a few of these cells bacilli have been found. In the spleen, where bacilli are distributed in fairly large numbers, leucocytes are scanty.

In Fowl 19, which died 14 days after intravenous inoculation with 10 mg., the distribution of bacilli is the same as in Fowl 21, and the only difference noted with regard to the leucocytes and lymphocytes is that the former cells are less numerous both in the lungs and liver. No bacilli have been found within leucocytes.

In Fowl 82, which died 53 days after intravenous inoculation with 10 mg., the lungs contain many caseous, circumscribed tubercles in which bacilli are plentiful; at the periphery of these lesions oxyphil leucocytes are numerous, and masses of these cells occur in the interior of some of the tubercles; there is a moderate sprinkling of small lymphocytes at the periphery of the lesions. A few of the leucocytes contain bacilli. In the liver, where bacilli are rare, neither lymphocytes nor leucocytes are numerous, and these cells show no attraction towards the bacilli. At the periphery of a circumscribed caseous tubercle found in the kidney a few leucocytes are present, but there appears to be no infiltration of small lymphocytes.

In Fowl 136, which died 59 days after intravenous inoculation with 20 mg., the lungs contain many minute, partially necrotic foci, and in these bacilli are fairly numerous; the liver is in a similar condition. In both lungs and liver leucocytes and lymphocytes are not greatly in excess of the normal. No bacilli have been found within leucocytes.

In Fowl 80, which was killed 102 days after intravenous inoculation with 1 mg., the lungs contain many caseous circumscribed tubercles where bacilli are numerous; both leucocytes and lymphocytes are rather scanty, but some are present at the periphery of the lesions. No bacilli have been found within leucocytes. In the lesions found in the liver, where bacilli are rare, leucocytes are abundant and lymphocytes are fairly numerous.

In Fowl 154, which was killed 115 days after intravenous inoculation with 1 mg., the liver contains many lesions (some of them caseous) in which bacilli are moderately numerous. Small lymphocytes are very abundant and form a broad periphery round the caseous patches; oxyphil leucocytes are less numerous but occur in groups here and there, showing no very close relationship to the bacilli. No bacilli have been found within leucocytes.

Plasma Cells.

The larger lesions, which are caseous in the centre always possess a well marked peripheral zone, and in this zone fibroblasts are generally numerous. Amongst these cells there is often a sprinkling of plasma cells, some of which are narrow and elongated. The lungs of Fowls 80 and 82 afford good examples of this condition. And where there is an abundant infiltration of small lymphocytes, *e.g.* in the livers of Fowls 21 and 154, many cells also occur which react to Pappenheim's stain; but these generally resemble large lymphocytes and are less frequently of the compressed or elongated type. With the small lesions which are only slightly necrotic and have no definite boundary zone plasma cells are not associated. These cells are also absent in situations which contain bacilli but show no evidence of tissue destruction or proliferation.

Endothelial and Parenchymatous Cells.

In the small necrotic or partially necrotic foci these cells merely show degenerative change. In the larger lesions, which are completely necrotic in the centre, the cellular zone bordering on the necrotic material sometimes contains nuclei in process of amitotic division, and collections of nuclei apparently formed in this way are seen, but they do not form typical giant cells. The cells from which these nuclei have been derived are probably endothelial or parenchymatous. The lungs of Fowl 80 afford good examples of aggregations of nuclei surrounding caseous patches.

Fibroblasts.

There is a well marked tendency for all except the smallest caseous patches to be surrounded by a complete and dense layer of fibroblasts. The greater number of these cells do not react to Pappenheim's stain and are probably derived from pre-existing fibrous tissue.

Summary.

Evidence of dissemination has only been found, with minor exceptions, after intravenous inoculation. After this method of infection also the bacilli sometimes disappear completely. When they survive they are found most frequently in the lungs; here they produce discrete tubercles, in the breaking down centres of which the bacilli sometimes appear to have multiplied since their arrival. In the liver and spleen minute and often numerous deposits of bacilli are found; but the bacilli are generally fragmentary; they have not been found to show definite signs of multiplication in these organs.

With regard to the tissue cells I may, for the sake of avoiding lengthy repetition, express my results in connection with what I have recorded on a later page concerning the reaction of the fowl's tissues to the avian bacillus. In certain respects the conditions are the same. In infections with mammalian bacilli, no new features of importance have been noted regarding the relations of leucocytes, lymphocytes, and plasma cells. No bacilli have been found within leucocytes. Plasma cells, including the elongated forms, are sometimes conspicuous at the periphery of the lesions. The only difference noted regarding the endothelial and parenchymatous cells is that when these cells degenerate and lose their individuality their nuclei, though showing a tendency to divide amitotically do not, in the case of infection with mammalian bacilli, multiply so abundantly as in some of the chronic lesions produced by avian bacilli. Where the mammalian bacilli have produced lesions of any considerable size there is always formed a well marked peripheral zone of fibrous tissue. This is a conspicuous feature in the lungs, where mammalian bacilli produce more tissue reaction and larger lesions than avian bacilli.

III.—THE CULTURAL AND MICROSCOPIC CHARACTERISTICS OF THE AVIAN BACILLI.

I am indebted to my colleagues for the primary cultures.

In order to obtain good growths I have found it necessary to employ a glycerinated medium. I have used glycerinated serum, agar, potato, and broth, and have prepared each of these media in the same way as for the growth of the mammalian bacillus.

Regarding cultural characters, I have found that the avian bacillus grows well, at 37½° C., on glycerinated serum, agar, and potato, but differs markedly from the mammalian bacillus in the slimy, putty-like appearance of the growth. The growth on potato generally assumes a yellow or brown pigmentation. When the bacilli are allowed to grow down a sloped surface of glycerin-serum on to a layer of broth, none of the viruses which I have examined have spread over the broth in the form of a surface pellicle; they have fallen down to the bottom of the flask and produced a powdery growth at the bottom of the broth. But I have found in some instances that, by keeping the bacilli in contact with the surface of the broth for a long time, a surface pellicle could be obtained.

In general cultural characteristics I have observed no differences between the various avian viruses which I have examined.

I have also investigated the microscopic characters of the bacilli.

In films obtained from cultures it is noticeable that the avian bacillus emulsifies readily and does not present the solid clumps of closely adherent bacilli which are characteristic of the mammalian bacillus.

The bacilli grown on glycerin-serum are generally very short (·5 to 1 μ) and rather thick, often looking like cocci. Other media commonly yield longer and more irregular forms; and such forms are also sometimes found on glycerin-serum. Amongst these irregularities is found every counterpart to the irregularities observed amongst mammalian bacilli; and, in addition, large club-shaped thickenings are common and branching occurs more frequently than with the mammalian bacillus.

When growing freely in the tissues the avian bacillus is short, straight, and uniformly stained; when growing under difficulties it is longer and more irregularly stained and is indistinguishable from mammalian bacilli growing under similar adverse conditions.

IV.—THE HISTOLOGICAL CHARACTERISTICS OF INFECTIONS WITH THE AVIAN VIRUSES.

A.—IN FOWLS.

Dissemination of Bacilli.

After Feeding.

After this method of experimental infection very few bacilli are found in the lungs and sometimes none at all; occasionally, though no bacilli are demonstrable, a few minute lesions are found which may be attributable to bacilli formerly present. The condition of the liver varies in different cases; bacilli may be absent; they may be distributed about the tissue in small numbers; or they may be plentiful and form small groups and colonies; and when bacilli are scanty there may be many lesions probably attributable to bacilli which have disappeared. No bacilli or lesions suggestive of bacilli have been found in the kidneys. In the spleen, bacilli may be absent, scanty, or fairly numerous; and caseous lesions may be present which have probably been caused by tubercle bacilli though none are demonstrable. There is dense thickening and caseation of the intestinal walls, wherein bacilli are frequently numerous.

After Subcutaneous Inoculation.

In the lungs, bacilli may be absent, scanty, or abundant. In the liver they are usually generally distributed and occur in large numbers. None have been found in the kidneys, but in one specimen there are a few minute lesions which may have been caused by tubercle bacilli. In the spleen, bacilli are usually very abundant, and sometimes have grown into dense masses of colonies.

After Intraperitoneal Inoculation.

The fate of bacilli introduced by this method appears to be uncertain. In Fowl 164 no bacilli were found in the lungs or kidneys, but they occurred in dense masses in the liver and spleen. In Fowl 298 no bacilli were found in the lungs, liver, kidneys, or spleen, but a few small foci, possibly due to tubercle bacilli, were observed in the lungs and liver.

After Intravenous Inoculation.

In the lungs bacilli are always numerous and sometimes form colonies in all parts of the tissue. In the liver and the spleen it is always found that the whole of the tissue is riddled with dense masses of bacilli. The kidneys are less affected than any of the other organs, but bacilli occur in small groups or colonies which may be either scanty or fairly numerous. Similarly it is found that after intravenous inoculation of dead bacilli, *e.g.*, in Fowl 392 inoculated with 50 mg., a more toxic effect was produced upon the liver and spleen than upon other organs.

Tabular Statement.

The data on which the above summary is based exhibited in the following table.

DISSEMINATION OF AVIAN BACILLI IN THE FOWL.

Mode of Infection.	Duration of Experiment in Days.	Virus.	Dose.	Number of Animal.	Presence or Absence of Bacilli in Various Organs.			
					Lungs.	Liver.	Kidneys.	Spleen.
Feeding	63	P XXVII.	1 glyc. serum culture.	40	1 T.B. found. A few minute lesions.	Absent. One doubtful lesion.	Not examined	Absent.
Feeding	92	P XXI.	10 mg.	26	Absent. A few minute doubtful foci.	A few bacilli. Many lesions.	Absent	Bacilli rare. Caseous lesions.
Feeding	96	P III.	1 glyc. serum culture.	36	A few; fragments.	Small groups.	Absent	Absent.
Feeding	103	P XXI.	10 mg.	28	Absent	A few	Not examined	A few.
Feeding	107	P II.	2 glyc. serum cultures.	32	Absent. A few doubtful foci.	Absent. Some lesions.	Absent	Fairly numerous.
Feeding	131	A I.	10 mg.	146	Absent	Very scanty	Absent	Bacilli rare. Caseous lesions.
Feeding	162	A X.	Small	264	Absent	Scanty	Absent	Scanty.
Subcutaneous	47	A VII.	10 mg.	220	Absent	Moderate numbers.	Absent	Scanty.
Subcutaneous	61	P II.	10 mg.	162	Absent	Numerous	Absent	Abundant.
Subcutaneous	71	P XXI.	10 mg.	148	A few	Generally distributed, but no clusters.	Absent. A few minute lesions.	Numerous; many small groups.
Subcutaneous	108	P II.	1 mg.	158	Abundant	Abundant	Absent	Abundant.
Intraperitoneal	61	P II.	10 mg.	164	Absent	Large masses	Absent	Dense masses.
Intraperitoneal	81	A IV.	20 mg.	298	Absent. A few doubtful foci.	Absent. Small lesions.	Absent	Absent.
Intravenous	29	A II.	0.1 mg.	92	Numerous small colonies.	Dense masses	A few in vessels.	Dense masses.
Intravenous	33	P III.	1 mg.	100	Scattered; some colonies.	Dense masses	One small group.	Dense masses.
Intravenous	35	A IV.	50 mg.	108	Numerous colonies.	Dense masses	Several groups	Dense masses.
Intravenous	37	A II.	10 mg.	96	Abundant colonies.	Dense masses	Several small colonies.	Dense masses.
Intravenous	42	P XXI.	1 mg.	152	Many small colonies.	Dense masses	Several small colonies.	Dense masses.
Intravenous	56	P XLIV.	0·01 mg.	172	A few small lesions, containing many bacilli.	Dense masses	Absent	Dense masses.

Destruction, Arrest, and Multiplication of Bacilli.

In the Subcutaneous Tissue.

In the local lesion of Fowl 220, 47 days after subcutaneous inoculation with 10 mg., there are large caseous areas and also small circumscribed tubercles in proximity to the subjacent muscle. Bacilli are very scanty, and nowhere form groups.

In the local lesion of Fowl 148, 71 days after subcutaneous inoculation, there are large necrotic areas and also many caseous, circumscribed tubercles. Bacilli are scattered about in all parts of the specimen, and often occur in small groups. Some of the bacilli are fragmentary and others are very long, much longer than serum-grown bacilli. There are no large groups or colonies, and it appears that, though some of the bacilli have retained their vitality and been able to grow, growth does not take place readily or abundantly.

In the Lungs.

Though the lungs are not readily affected except by intravenous inoculation, when the avian bacillus arrives in considerable numbers in this organ it usually survives and multiplies. In all the cases of intravenous infection which I have examined, colonies of bacilli are found scattered throughout the lung tissue. But the presence of bacilli is associated with remarkably little tissue change. There is no tendency to consolidation, and the foci which are laden with bacilli, though very numerous, show little tendency to become confluent. The bacilli are short and straight, and occasionally show a tendency to form rosettes.

In the Liver.

The fowl's liver is highly susceptible to the avian bacillus. When the number of bacilli which reach the organ is very small, it is probable that some of them fail to survive; and in cases of relatively slight infection lesions may be found which are evidently due to the tubercle bacillus, though no bacilli are present in them. But usually, and in all cases where the infection is severe, the bacilli multiply rapidly and very abundantly, forming dense colonies and rosettes in all parts of the tissue.

In the Kidneys.

The kidney appears to be one of the least favourable sites for the development of bacilli. Amongst the specimens examined, no bacilli have been found except in the animals inoculated intravenously. Most of these show bacilli, which have formed small groups or colonies, but the number of bacilli present is very much smaller than in the lungs, and, compared with the liver, the amount of dissemination is extremely small. The avian bacillus is usually a very small organism, and it seems probable that when it arrives in the kidneys by the blood-stream it is readily excreted.

In the Spleen.

This is a very favourable site for the deposit and development of bacilli. As in the liver, they grow very luxuriantly, forming colonies and rosettes throughout the spleen substance.

In the Alimentary Tract.

Along the course of the alimentary tract tubercular lesions with greatly thickened walls and caseous centres are usually abundant and contain many bacilli.

In the Blood-Vessels.

In all the animals which have been severely infected (by intravenous inoculation) bacilli have been found in the blood-vessels at the termination of the experiment (the duration of the experiments ranging from 29 to 42 days). In many of the specimens intravascular bacilli are fairly numerous; they are loosely distributed, never occur in clumps, and are generally not attached to or within cells. The readiness with which intravascular bacilli can be found in sections indicates that the number in the circulation must be very large

Reaction of the Tissue Cells.

Leucocytes and Small Lymphocytes.

Fowl 40.—In the specimen of lung, where only one bacillus has been found, there are a few small foci which are filled up with leucocytes and small lymphocytes and are probably due to the former presence of small numbers of bacilli. In the liver there is a slightly caseous area containing large numbers of leucocytes and lymphocytes, and in other places there is a leucocytic infiltration which is particularly well marked at the periphery of blood-vessels; no bacilli have been found in the specimen, but the caseous area may undoubtedly be regarded as a tubercle, and possibly the infiltration which is found elsewhere has been caused by tubercle bacilli formerly present.

Fowl 26.—The liver of this animal is a good example of a mild, retrogressive infection. In only one lesion have a few bacilli been found, but many other lesions are present which seem undoubtedly due to the former presence of tubercle bacilli. The lesion where the bacilli have been found contains very large numbers of oxyphil leucocytes but no lymphocytes; in the other lesions lymphocytes are always present as well as leucocytes, the lesions being generally filled up with groups of these two types of cells. There is also a well marked zone of leucocytes and lymphocytes surrounding the walls of the blood-vessels. In addition to the ordinary finely granular leucocyte there are many masses of cells which are rather larger and are filled with larger and more deeply staining granules. The spleen contains many discrete tubercles which are centrally caseous and surrounded with fibroblasts; they contain few bacilli. Leucocytes are abundant throughout the tissue and masses of broken down leucocytes are found amongst the caseous material of some of the tubercles.

Fowl 36.—The liver contains caseous, circumscribed foci in which bacilli are moderately numerous. Leucocytes are fairly plentiful just within the periphery of the lesions, but none have been found to contain bacilli.

Fowl 28.—In the liver there is a marked leucocytic infiltration, particularly around the blood-vessels and between the lobules; a few tubercle bacilli occur, but there are no definite tubercles.

Fowl 162.—The liver is crowded with discrete tubercles which are centrally caseous; bacilli are numerous in the lesions but have not grown into rosettes; leucocytes are fairly numerous at the margin of the lesions, and one or two have been found to contain bacilli.

Fowl 148.—In the local lesion, which is extensively caseous, bacilli occur in small groups but do not form dense colonies; in the areas not completely necrotic there is a moderate sprinkling of oxyphil leucocytes, and a few of these contain bacilli; small lymphocytes are rather more numerous than the leucocytes but do not form dense groups. The liver contains innumerable caseous tubercles in which bacilli are scattered but do not form groups or colonies; both leucocytes and lymphocytes are found at the periphery of the lesions but not in very large numbers; no bacilli have been found within leucocytes. In the spleen, which is in the same condition as the liver but rather more advanced, a few of the leucocytes contain bacilli. In the kidneys there are a few small foci which are probably minute tubercles and have been overrun with lymphocytes; no bacilli have been found.

Fowl 158.—The liver is very extensively caseous and bacilli are abundant; oxyphil leucocytes are very numerous in all parts of the tissue and are collected into groups both in the caseating and in the non-caseous areas; several leucocytes have been found containing bacilli.

Fowl 92.—The liver is occupied with enormous numbers of bacilli which have grown everywhere into colonies but have produced relatively little tissue destruction; leucocytes are notably rare, and none have been found containing bacilli; small lymphocytes occur in fairly large numbers amongst the cells between the groups of bacilli but are not packed in close masses.

Fowl 100.—The lungs contain several small colonies of bacilli; bacilli are also scattered loosely over all parts of the tissue; the bacilli are very rarely found within leucocytes, though these cells are very plentiful throughout the tissue and particularly at the periphery of groups of bacilli. The liver is crowded with dense colonies of bacilli; oxyphil leucocytes are plentiful and a few of them contain bacilli; between the clusters of bacilli small lymphocytes are fairly numerous. The spleen is in the same condition as the liver.

Fowl 108.—In the lungs groups and colonies of bacilli are abundant but there is relatively little tissue change; oxyphil leucocytes are scanty, and none have been found containing bacilli. The liver and spleen resemble those of Fowl 100.

Fowl 96.—The whole of the lung tissue is sprinkled with colonies of bacilli which have produced no appreciable histological change; both leucocytes and lymphocytes are scanty. The liver and spleen, which are packed with colonies of bacilli, contain relatively few oxyphil leucocytes but show a moderate degree of infiltration with small lymphocytes.

Fowl 152.—In the lungs there are many small colonies of bacilli, but there is very little tissue change. The liver contains abundant colonies of bacilli between which there is a copious infiltration of lymphocytes and leucocytes.

Plasma Cells.

Where there is an abundant infiltration of small lymphocytes, which is frequently noticeable in the livers of severely infected fowls, cells which react to Pappenheim's stain are also numerous, but generally resemble large lymphocytes in shape and are not commonly of the compressed or elongated type. No plasma cells have been found in situations which contain bacilli but show either no evidence of tissue destruction or only slightly necrotic foci with no definite boundary zone. Moreover, at the periphery of the larger lesions, which show a well marked boundary of fibroblasts, plasma cells have not been found in any of the specimens examined. There is also an absence of these cells at the periphery of blood-vessels situated amongst infected tissue.

Endothelial and Parenchymatous Cells.

In the minute lesions, which show little or no necrotic change, these cells show some protoplasmic degeneration, but no signs of proliferation. In the larger lesions, and particularly in the tubercles where caseation has set in, there is a tendency for the nuclei of these cells to divide amitotically. These newly-formed nuclei often form an irregular fringe at the margin of a caseous patch, and also frequently form large clumps which, taken together with the surrounding protoplasm, may be termed "giant cells"; but their protoplasmic matrix is much too imperfectly defined to justify the assumption that they are individual, multinucleate cells.

Fibroblasts and Degenerative Change.

At the periphery of lesions showing caseous change there is a well-marked tendency to the formation of a fibrous layer, composed of cells which do not react to Pappenheim's stain, but are apparently derived from pre-existing connective tissue corpuscles. There is also a tendency, particularly well marked in lesions not centrally caseous but containing large masses of bacilli, for the formation of a peripheral deposit of concentric layers of hyaline material. And in lesions partially caseous and less densely packed with bacilli, globules of a similar hyaline material frequently occur in the necrotic areas.

Summary.

After feeding, dissemination of bacilli beyond the region of the alimentary tract is relatively scanty. Very few bacilli find their way to the lungs, and none have been found in the specimens of kidneys. More bacilli find their way to the liver and spleen, and in these organs the bacilli may be generally distributed in small numbers, or may even form small groups and colonies, though in other cases the number of bacilli present is scanty. After subcutaneous inoculation the dissemination of bacilli, though not always profuse, is less restricted than after feeding; the liver and spleen receive much larger numbers of bacilli than the lungs and kidneys. After intraperitoneal inoculation the results are variable; bacilli may be found in very large numbers in the liver and spleen.

When the bacilli are introduced into the subcutaneous tissue, the majority of them are detained *in situ* and give rise to extensive tubercles with broad cellular walls; and after feeding the bacilli are detained in a similar manner within lesions formed in proximity to the alimentary tract. In no instance have the lungs been found to be severely diseased, and frequently either only few or no bacilli are to be found; where bacilli are numerous and have grown into colonies, which is particularly the case after intravenous inoculation, they have produced remarkably little tissue damage. The fowl's lung is often said to be highly resistant; this is true in the sense that the bacilli produce very little tissue damage; but it is a passive sort of resistance; the lung tissue does not destroy the bacilli, but allows them to pass through the pulmonary circulation without detention, and allows them to multiply, particularly in the septa between the pulmonary lobules, without hindrance. The high resistance of the kidneys also appears to be of this passive nature, *i.e.*, the majority of the bacilli pass through the organ without detention and without provoking a tissue reaction. The liver and spleen are the two most susceptible organs, *i.e.*, the places wherein the bacilli lodge and find a particularly favourable culture medium. It is also important to note that bacilli are very frequently found within the blood-stream, and that this medium appears much less unfavourable to their existence than is the blood of mammals to the existence of mammalian bacilli.

Leucocytes and small lymphocytes are usually found in association with deposits of bacilli, but the latter cells do not come into close contact with them. The leucocytes are sometimes found to contain bacilli, but not very frequently; they do not play an important part as protective agents. They are most conspicuous in the chronic caseous lesions, where they form a broad zone at the periphery, not in contact with the caseous material containing bacilli, but amongst the external demarcation layer of fixed tissue cells. Plasma cells and large lymphocytes are often found at the periphery of the more chronic lesions, but play no part in attacking or ingesting tubercle bacilli. The avian bacillus is often carried along the circulation without attaching itself to the endothelial lining and without acting as an irritant to the endothelial cell. But it readily passes through these cells, particularly in the liver, and then commences to grow within the parenchymatous cells. In the cellular degeneration which follows, the cells lose their individuality, and there is then a tendency, particularly well marked in the more chronic lesions, for the nuclei present in these areas to multiply amitotically, forming very conspicuous and often circular masses of nuclei embedded in a protoplasmic matrix. Another feature of the chronic avian tubercle with a caseous centre is the formation of a very dense fibrous periphery. But in tissues where the deposits of bacilli multiply rapidly and very profusely, there is an absence of tubercle formation; often a large growth of bacilli evinces little or no tissue reaction, but not infrequently these areas of bacilli are surrounded by concentric layers of hyaline deposit.

The most striking feature about these results is that, under different circumstances, the same bacillus produces in the fowl widely different types of disease. On the one hand there is the slowly progressive caseous tubercle with a well defined fibrous periphery, within which bacilli are generally scanty and never very abundant; and on the other hand there is an enormous profusion of bacilli with no tubercle formation and very little tissue damage.

As the caseating lesions are undoubtedly due to the action of the tubercle bacillus, there is thus the paradoxical result that a few bacilli produce a greater amount of tissue damage than a large number. In explanation of this paradox, the first point of difference to note is that in the caseous type of lesion many of the bacilli break down and disappear, whereas in the other type of disease the bacilli grow profusely and show no sign of disintegration. The caseation must therefore be associated with the disintegration of bacilli and the escape of their contents into the tissue. The question then arises, Why do the tissues cause the destruction of the bacilli in some cases but not in others? "Phagocytosis" does not afford any sufficient explanation, because there is no evidence that in the former case any particular type of cell attacks the bacilli more vigorously than in the latter. It is, however, significant that this free multiplication of bacilli without tubercle formation is the effect commonly produced by intravenous inoculation, and is only found when bacilli have gained free access to the blood-stream. The difference, therefore, appears to be associated with some change in the plasma, whereby the cells nourished with normal plasma prove a less favourable medium for the growth of the bacillus than cells nourished with plasma which has been modified by the circulation of numerous bacilli. This influence must be primarily on the cells rather than on the bacilli, because it would be unreasonable to suppose that circulation in the blood-stream, *per se*, endowed the bacilli with greater capacity for growth. It is also noteworthy that the bacilli derive more nourishment, as evinced by their more abundant growth, from tissue where disintegration is only slight than from tissue which has been reduced to a homogeneous admixture of nuclear with protoplasmic material. This may be associated with the inability of the bacilli to grow within the nuclei of cells.

As I have already pointed out, the two organs most readily attacked are the liver and spleen; and in these two the bacilli grow in greatest profusion. The lungs and kidneys are much less susceptible, in the sense that much less tissue damage is found in these organs. But there is no evidence that either of these organs plays an important part in the destruction of bacilli After intravenous inoculation bacilli circulate freely through both organs and doubtless many are passed out in the renal excretion. It is also noteworthy that through the lungs, which must bear the initial brunt of the inoculation, the bacilli circulate without producing more than slight irritant effects. But they grow in the pulmonary tissue without hindrance; and in specimens which present no lesions to the naked eye numerous colonies are found, particularly in the connective tissue at the margins of the pulmonary lobules.

B.—IN RABBITS.

I.—Early Stages of Infection after Intravenous Inoculation with Avian Bacilli.

Dr. F. GRIFFITH inoculated a series of rabbits (Rabbits 1552–4, 1793, 1555, 1792, 1556) intravenously, the dose for each animal being 1 mg. of culture. One animal was killed at each of the following periods after inoculation:—24 hours, 3 days, 5 days, 6 days, 8 days, 11 days, and 14 days.

Lungs.

24 hours.—The effect of the inoculation is seen by a general distension of the alveolar walls and the presence of more leucocytes than normal in all parts of the tissue. There are many minute deposits of bacilli, but no clumps. The emulsion of culture appears to have been very finely divided. The bacilli have produced no definite tissue change; none have been found within leucocytes, nor do collections of leucocytes occur in situations where bacilli are present. In some of the larger blood-vessels which have been cut across, bacilli are present in small numbers; some of them are free, and others are attached to mononuclear cells.

3 days.—The general alveolar thickening is rather greater than in the former specimen; the leucocytes show a slight further increase, and the small lymphocytes are also rather more numerous. Bacilli occur singly, and a few small groups have also been found. There is no constant relation between the leucocytes and lymphocytes and the bacilli. Fairly large groups of both these types of cells occur amongst two of the larger collections of bacilli, but in other places these cells are not found in association with bacilli. No bacilli have been found within leucocytes. Both the endothelial and the epithelial cells are swollen, and some of their nuclei are of irregular outline; these conditions are not confined to situations where bacilli are present. Some of the bacilli are distinctly larger than serum-grown bacilli.

5 days.—The general thickening is rather more advanced than in the 3 days' specimen; the leucocytes show only a slight further increase, but the number of small lymphocytes has increased considerably. Bacilli are rather more frequent than in the last specimen, and in some of the small groups multiplication appears to have taken place. The small consolidated foci in which these groups of bacilli occur consist of greatly swollen epithelial and endothelial cells, together with many lymphocytes and a smaller number of leucocytes. No bacilli have been found within leucocytes.

6 days.—Very little change as compared with the last specimen, but a few bacilli have been found within leucocytes.

8 days.—Though some general thickening of the alveolar walls is still present, it is less in amount than in the last specimen. The general distribution of leucocytes and lymphocytes is the same as before. The sites in which bacilli occur are not more numerous, but there is more evidence of multiplication, many of the bacilli having grown into minute clumps. The condition of the lesions in which the groups of bacilli occur is the same as in the former specimen. The leucocytes appear to be only slightly attracted by the presence of bacilli, and very few have ingested them.

11 days.—The tissue is much more congested and shows a general thickening of the alveolar walls. Bacilli are still scanty, but occur in small groups of 5 or 6; none have been found within leucocytes.

14 days.—Compared with the last specimen, the lesions are much more numerous, and some of them are decidedly larger. There is also a further increase in the number of leucocytes and lymphocytes in all parts of the tissue. Bacilli are distributed more generally, and the number of small clusters is greater. Some of the lesions show a slight tendency to caseation, and there is an occasional increase in the nuclei of some of the epithelial and endothelial cells found in these lesions. Though leucocytes are numerous, none have been found which contain bacilli.

Liver.

24 hours.—Bacilli are scattered about the tissue in small numbers; they occur either singly or in groups of not more than two or three. They are found sometimes within endothelial cells and sometimes within liver cells; the nuclei of the cells containing them are often somewhat distorted. No other tissue changes are observable. Leucocytes are not in excess of the normal, and very few have been found containing bacilli.

3 days.—Perhaps the bacilli occur a little more frequently than in the former specimen, but the difference is only slight. They occur in the same situations, and no further histological changes are observable in the liver cells and endothelial cells which contain bacilli. It is, however, noticeable that many of the bacilli in these situations, though they have not multiplied, have distinctly increased in length. Leucocytes are scanty, but in one situation a small group of these cells has been found surrounding and ingesting a group of bacilli.

5 days.—Bacilli occur more frequently both within liver cells and endothelial cells. They also show signs of multiplication in these situations. Usually the increase is only small, but one liver cell has been found the nucleus of which stains diffusely, and is completely surrounded by a ring of bacilli. There is no increase in the number of leucocytes; one of these cells has been found containing bacilli. The number of small lymphocytes is rather greater than in former specimens. The amount of histological change in the tissue is extremely small, and would hardly be noticed in specimens not stained for bacilli.

6 days.—The foci in which bacilli occur have not increased in number; but, in most situations where they occur, the bacilli have increased in number and show a tendency to ring formation. The small groups of bacilli have produced definite lesions, characterised by protoplasmic disintegration of the parenchymatous cells, swelling and increase of the surrounding endothelial cells, and some infiltration of lymphocytes. Leucocytes are not very numerous, and rarely contain bacilli.

8 days.—Compared with the last specimen, the amount of pathological change shows a small but appreciable increase. The sites where bacilli occur are rather more numerous, and the groups of bacilli are slightly larger. There is a little increase in the number of leucocytes, and also of lymphocytes. A few of the endothelial cells show nuclear changes preparatory to mitosis. Round one or two of the larger groups of bacilli there is a small collection of lymphocytes and leucocytes. A few leucocytes contain bacilli.

11 days.—Bacilli are very numerous and have grown into rosettes and small colonies in almost every microscopic field. There is also a much denser infiltration of lymphocytes and leucocytes in many of the regions where bacilli are abundant. The leucocytes exhibit only slight phagocytic activity.

14 days.—There is a very great increase in the number of bacilli, which are now found plentifully in almost every microscopic field (oil-immersion lens). They have multiplied into groups, and often form typical rosettes. The interior of the liver cells is the usual place in which multiplication has occurred. The protoplasm of many of these cells is crowded with bacilli, and their nuclei have increased by direct division. Sometimes the bacilli form a ring surrounding the nuclei, and sometimes the nuclei are displaced to one side of the cell; occasionally, where two or three liver cells have fused together, there is a confused mass of nuclei and bacilli. No mitotic figures have been found in liver cells containing bacilli, but in adjacent liver cells mitoses have been found, though not very commonly. The presence of bacilli within endothelial cells is relatively rare, their preference for the liver cells being much more strongly marked in this than in earlier specimens. There is no appreciable increase in the lymphocytes, but the leucocytes are distinctly more numerous than in former specimens. In a few places a small group of leucocytes is found attacking and ingesting bacilli but this is exceptional; the great majority of the leucocytes do not contain bacilli, and these cells are not more numerous in proximity to bacilli than in other situations. The most striking features of the specimen are the multiplication of bacilli within liver cells, and, with the exception of the protoplasmic disintegration of these cells, the complete absence of tissue destruction.

Kidneys.

24 hours and 3 days.—No lesions or bacilli have been found.

5, 6, 8, and 11 days.—Two or three bacilli have been found in the first specimen but none in the others.

14 days.—A few bacilli have been found within blood-vessels but none elsewhere.

Spleen.

24 hours.—Bacilli are scattered about the specimen in rather larger numbers than have been found in any of the other organs at this period. A few of the bacilli are longer than serum-grown bacilli. Leucocytes are numerous throughout the specimen and also occur in groups, where many of them show a granular disruption of their nuclei. It is doubtful whether the tubercle bacilli have caused this change, as these groups of leucocytes are not associated with collections of tubercle bacilli. Some of the leucocytes are close to bacilli, but there is only slight evidence of phagocytosis.

3 days.—Bacilli are slightly more numerous than in the last specimen and a few have been found within blood-vessels. Occasionally a small group of bacilli is found which appears to have increased by multiplication. Leucocytes are abundant; they appear to be attracted towards the bacilli, and bacilli are sometimes found amongst their granules. Bacilli are frequently present within endothelial cells.

5 days.—The deposits of bacilli are numerous and there is further evidence of multiplication, many of the newly formed groups occurring within endothelial cells. There is a further increase in the number of leucocytes. Amongst the groups of bacilli these cells are often plentiful; they contain bacilli not unfrequently and many of them shows signs of nuclear disintegration. A few bacilli have been found within blood-vessels.

6 days.—No appreciable change as compared with the last specimen.

8 days.—There is a further increase in the number of bacilli, and the colonies have become rather larger. Many of the colonies have grown into solid balls, but some are more loosely arranged and show a tendency to rosette formation; no completely formed rosettes have been found. Leucocytes are even more numerous than in the last specimen and are often found surrounding and ingesting bacilli. The number of actively phagocytic leucocytes is much larger than in any previous specimen. Although the number of bacilli present in the tissue is very large, there is a remarkable absence of tissue destruction.

11 days. — The bacilli have increased in number, but there is a diminution in the phagocytic activity of the leucocytes.

14 days.—There is a further increase of bacilli. The colonies are larger and rosette formations are more definite. The nuclei of the endothelial cells are greatly swollen, and some of them have multiplied by direct division, without division of the protoplasm. These collections of nuclei are surrounded by bacilli, but are not massed together in sufficiently large numbers to form conspicuous giant cells. The protoplasmic material surrounding these nuclear groups stains imperfectly but is not definitely caseous. In the areas where bacilli are abundant the leucocytes show a marked falling off in number and do not contain bacilli; but in the less affected parts of the tissue these cells are numerous, are often found in contact with small groups of bacilli, and sometimes contain bacilli amongst their granules.

II.—Early Stages of the Infective Process in Rabbits after Subcutaneous Inoculation with Avian Bacilli.

Dr. F. GRIFFITH inoculated a series of rabbits subcutaneously, over the scapula, each with a dose of 50 mg. of avian culture. One animal was killed at each of the following intervals after inoculation:—24 hours, 3 days, 5 days, 8 days, and 14 days.

Twenty-four Hours.

The mass of bacilli (Rabbit 1642) is fairly compact and has only penetrated to a slight extent into the subjacent muscle. Amongst the bacilli there are a few long, thick forms; these may have been produced after the inoculation; they are not usually met with in recent serum cultures. The deposit of culture is infiltrated with enormous numbers of leucocytes. In the central areas the leucocytes have completely lost their granulation and are crowded with bacilli. But where the bacilli are more loosely distributed, though still very numerous, most of the leucocytes retain their granulation; the majority of these contain bacilli in their interior but some of them do not, although there are large numbers of free bacilli close at hand. On passing to areas where the bacilli are more scantily distributed, the percentage of leucocytes not engaged in phagocytosis rapidly increases, and many of the bacilli are found lying free. Outside the area of bacilli leucocytes are still plentiful and appear to be converging towards the central foci. The capillaries found in the adjacent muscle contain numerous leucocytes but no bacilli.

No bacilli have been found in the specimens of lung, liver, and kidney; but one small group has been found in an axillary gland.

Three Days.

In the local lesion (Rabbit 1643) some of the bacilli are broken up, and a few show signs of beading. The relation of leucocytes to the bacilli is the same as in the previous specimen. In areas containing large numbers of bacilli many of the leucocytes have been able to retain their granules, but there are also large numbers of loosely scattered granules; this free distribution of loose granules is more noticeable than in the previous specimen. No bacilli have been found within blood-vessels.

No bacilli have been found in the specimens of lung, liver, and kidneys. There are a few particles of acid-fast material in the spleen which might be fragments of bacilli. One small cluster of bacilli has been found in an axillary gland.

Five Days.

Amongst the central mass of bacilli in the local lesion (Rabbit 1644) many are broken up; many long, thick forms are present; and a considerable number have lost, or partially lost, their acid-fast properties and take on a more or less definite blue stain. Most of the leucocytes in this central area have lost their granulation. Passing outwards, there is found, as in the earlier specimens, a dense mass of leucocytes which retain their granulation, and there are also large numbers of loosely scattered granules. In the loose areolar tissue adjacent to the lesion the most striking feature is the large number of endothelial cells and fibroblasts which have taken up bacilli. The protoplasm of many of these cells has been replaced by a solid mass of bacilli, which, from their compact arrangement and normal size and staining properties, appear to have multiplied in these situations. Leucocytes are numerous in this part of the tissue, but in contrast to the endothelial and connective tissue cells they rarely contain bacilli. A few bacilli have found their way between the muscle fibres, and in this situation also they are generally contained within the fixed tissue cells. No bacilli have been found within the blood-channels. Though the oxyphil leucocytes still constitute almost the whole of the cellular infiltration, a few small lymphocytes have made their appearance at some parts of the periphery.

No bacilli have been found in the specimens of lungs, liver, kidneys, and spleen; but a few occur within an axillary gland.

Eight Days.

Compared with the last specimen the main difference in the local lesion (Rabbit 1645) is that it has spread much more extensively into the surrounding areolar tissue. The meshes of this tissue are greatly thickened, owing to an increase of the endothelial and connective tissue elements and also to an infiltration of leucocytes and lymphocytes. Many of the fixed tissue cells contain bacilli; but the leucocytes are also active, and it is noticeable that in these outlying regions a greater proportion of leucocytes contain bacilli than was the case in the corresponding situations in the five days' specimen. Another change noted in the peripheral areas is an increase in the number of lymphocytes. The blood capillaries are dilated and more numerous than before; their endothelium is also more swollen, but no bacilli have been found actually within their lumen. Numerous lymphocytes, as well as leucocytes, are present within the blood-channels. The central parts of the lesion still contain very numerous bacilli and show more necrotic change; they are a confused mass of broken down cells, in which very few of the leucocytes present have retained their granules.

No bacilli have been found in the specimens of lung, liver, kidney, or spleen. In an axillary gland, bacilli are scattered in small numbers.

Fourteen Days.

The local lesion (Rabbit 1646) shows more advanced necrotic areas and a decrease of the number of bacilli in these situations. The number of leucocytes also shows a diminution, whilst the tissue outside the necrotic areas shows an increase both of fibroblasts and of lymphocytes. Some of the blood-capillaries are cut off amongst necrosing tissue and appear to be breaking down.

No bacilli have been found in the specimens of lung or kidney. One bacillus has been found in a minute lesion in the liver and one in the spleen. In a scapular gland there is extensive necrotic change; bacilli are abundant, and have commenced to form rosettes.

III.—Terminal Results of the Infection of Rabbits by Avian Tubercle Bacilli.

Dissemination of Bacilli.

After Feeding.

When the dose is small (from 1 to 20 mg. of culture) this appears to be a relatively mild method of infection. A few minute foci, in which bacilli occasionally occur, may be found in the lungs; in the liver, caseous tubercles of a chronic type have been found, but bacilli are scanty; in the kidneys a few caseous tubercles may be found, and many contain rather more bacilli than corresponding lesions in the liver. In no situation have large deposits of bacilli been found.

After Subcutaneous Inoculation.

In the lungs consolidated or caseous foci are often present, but these are usually small and do not contain many bacilli; in only one specimen has a nodule been found which contains bacilli in large numbers. In the liver small lesions are usually numerous, but do not contain many bacilli. The kidneys are frequently affected, and sometimes fairly large caseous lesions are found, but on the whole bacilli are scanty, though more numerous than in the liver. In some of the lymphatic glands bacilli may be found in very large numbers. On the whole, therefore, bacilli are disseminated more freely than after feeding, but do not invade the organs in large numbers.

After Intraperitoneal Inoculation.

In the lungs bacilli are usually distributed in large numbers. They are extremely abundant in the liver, spleen, and lymphatic glands; they have always been found in the kidneys, but in this organ their number is relatively small, being on the whole rather less than the number present in the lungs. It will be seen from these results that the intraperitoneal method of infection is followed by much more abundant dissemination than either of the two former methods.

After Intravenous Inoculation.

General dissemination follows inoculation by this method, and unless the dose is very small bacilli are found in large numbers in all the organs except the kidneys, where they are relatively scanty. After a minute dose (·001 mg.) general dissemination occurs, but the bacilli tend to be localised in caseous nodules, and do not attain to the enormous numbers found after inoculation with a larger dose (1 mg.).

Tabular Statement.

The following table exhibits the above results in detail:—

DISSEMINATION OF AVIAN BACILLI IN THE RABBIT.

Mode of Infection.	Duration of Experiment in Days.	Virus.	Dose.	Number of Animal.	Presence or Absence of Bacilli in Various Organs.			
					Lungs.	Liver.	Kidneys.	Other Organs Examined.
Feeding	91	A VII.	10 mg.	1282	Absent	1 T.B. found	Absent	Absent in spleen. Scanty in intestinal walls.

DISSEMINATION OF AVIAN BACILLI IN THE RABBIT—*continued.*

Mode of Infection.	Duration of Experiment in Days.	Virus.	Dose.	Number of Animal.	Presence or Absence of Bacilli in Various Organs.			
					Lungs.	Liver.	Kidneys.	Other Organs Examined.
Feeding	163	A I.	1 mg.	1090	Absent	Absent	Absent	Absent in a mesenteric gland.
Feeding	195	P XXI.	20 mg.	1092	Rare	Rare	Fairly numerous.	
Feeding	323	P XLIV.	10 mg.	1180	Rare	Bacilli absent. Small caseous lesions.	A caseous tubercle containing several bacilli.	
Subcutaneous	36	P II.	250 mg. of dead bacilli.	1541	One small group	Scattered in small numbers.	Absent	Absent in spleen.
Subcutaneous	52	A V.	10 mg.	956	Absent	Many lesions containing few bacilli.	Several lesions containing few bacilli.	Abundant in a bronchial gland.
Subcutaneous	69	P II.	5 mg.	326	Several lesions containing few bacilli.	Many lesions containing few bacilli.	Numerous	Dense masses in a mesenteric gland.
Subcutaneous	155	P XXI.	1 mg.	878	Scanty	Not examined	Rather scanty	Scanty but generally distributed in the spleen.
Subcutaneous	168	A V.	10 mg.	957	Abundant in one large lesion; scanty elsewhere.	Rare	Absent	Absent in spleen.
Intraperitoneal	13	P II.	50 mg.	294	Numerous	Dense masses	Scanty	Dense masses in the spleen and a mesenteric gland.
Intraperitoneal	22	P II.	1 mg.	292	Abundant small colonies.	Dense masses	Many small groups.	Dense masses in the spleen and a mesenteric gland.
Intraperitoneal	28	A V.	1 mg.	959	Scanty	Generally distributed, but only in minute groups.	Scanty	Abundant in a sternal gland.
Intraperitoneal	37	P XLIV.	1 mg.	1183	Moderately numerous.	Dense masses	Fairly numerous.	Dense masses in a mesenteric gland.
Intravenous	18	A X.	1 mg.	1791	Numerous	Dense masses	Rather scanty	Dense masses in spleen; numerous in a bronchial gland.
Intravenous	25	P XXVII.	1 mg.	775	Numerous	Dense masses	Scanty	Dense masses in a mesenteric gland.
Intravenous	36	P II.	50 mg. of dead bacilli.	1538	2 T.B. found	2 T.B. found	Absent	A few single bacilli in the spleen.
Intravenous	36	P II.	50 mg. of dead bacilli.	1539	Distributed in small numbers.	A few	Absent	Distributed in small numbers in the spleen.
Intravenous	36	P II.	10 mg. of dead bacilli.	1540	Absent	Very rare	Absent	Very rare in the spleen.
Intravenous	140	P XXI.	·001 mg.	880	Caseous nodules, some of which contain many bacilli.	Caseous nodules containing few bacilli.	Caseous nodules containing fairly numerous bacilli.	Moderately numerous in spleen.

Destruction, Arrest, and Multiplication of Bacilli.

In the Lungs.

In the specimens where no bacilli have been found, abnormalities, such as slight thickenings of the alveolar walls sometimes occur; but there are no tissue changes which definitely point to the former presence of tubercle bacilli; and from the general condition of the animals there has been no reason to suppose that many bacilli have previously been carried to the lungs.

In several specimens bacilli are present but scanty. Under these conditions the bacilli are associated with lesions which indicate that the reaction of the tissues has detained the bacilli *in situ*. In these lesions, which sometimes are no more than small collections of lymphocytes and leucocytes and sometimes are larger areas showing early caseation, bacilli may be found which stain irregularly and appear to be disintegrating, but more usually the bacilli are normal in appearance and, from their arrangement in twos and threes, appear to be slowly multiplying. And where bacilli are scanty in the lungs the condition of the other organs indicates that the lungs have not been likely to receive many bacilli from other sources. The conclusion appears to be that bacilli are not readily destroyed in the lungs, even when they only reach that organ in scanty numbers.

In a few cases the lungs contain discrete, extensively caseous nodules; and bacilli are confined to these situations. Rabbit 880, killed 140 days after intravenous inoculation with ·001 mg., is a good example. Here the small dose of bacilli originally conveyed to the lungs has been arrested and has set up chronic lesions within which the bacilli have multiplied. They do not, however, form the colonies of short, straight bacilli typical of luxuriant growth, but are often long and irregularly stained, an indication that they have been growing under difficulties.

Finally, the lungs sometimes contain large numbers of bacilli which are multiplying freely, are not limited to definite lesions, and have produced relatively little tissue destruction. This condition is always associated with dense multiplication of the bacilli in other parts of the body, and the condition of the lungs may be explained partly by a general lowering of the animal's resistance and partly by the frequency with which bacilli are being carried to the lungs from other sources.

In the Liver.

The rabbit's liver is very highly susceptible to the avian bacillus. In some cases, where the number of bacilli carried to the liver has probably been very small, suspicious lesions are found, though no bacilli are demonstratable. It is, therefore, possible that bacilli arrested in this organ may sometimes be destroyed. And after the less severe modes of infection lesions may be numerous in the liver but the number of bacilli in each may be scanty. It therefore appears that a certain amount of resistance has to be overcome before the bacilli can grow freely in this organ. But the usual result, in the severe infections, is to find that the bacilli have grown with extreme luxuriance; they appear, in histological specimens, as a pure culture grown in an eminently favourable medium.

In the Kidneys.

Bacilli are often arrested in the kidneys in the milder infections and invariably in the severer infections. In this organ it is usually found that they set up lesions of a chronic type, which after a certain stage begin to caseate. In many of these lesions bacilli are scanty, but in a few of the largest it is found that they have multiplied very considerably, though not forming dense colonies or rosettes. In some cases bacilli are irregularly distributed in the kidney without causing well-defined lesions and have frequently grown into small colonies; this condition is associated with the luxuriant growth of bacilli in other parts of the body. It appears, therefore, that when the general bodily resistance of the animal is lowered bacilli may grow more freely in the kidney and cause less tissue change. But it is also noteworthy that when other organs contain dense masses of bacilli the number of bacilli in the kidneys is always relatively small and may be scanty.

In the Spleen and Lymphatic Glands.

The lymphatic glands examined show that when the avian bacillus gains access to these organs it multiplies very abundantly. The spleen reacts very much in the same way as the liver; in severe infections it is crowded with dense colonies of bacilli.

In the Blood-Vessels.

In the milder infections bacilli are generally absent from the blood-vessels; but occasionally a few have been found in infections of a more or less intermediate type where bacilli are numerous in some organs but scanty in others: *e.g.*, a few free bacilli have been found in vessels of the lungs of Rabbit 326, though this tissue as a whole does not contain many bacilli.

In the more severe infections, where bacilli are abundant in the tissues, bacilli are readily found in the blood-vessels, either singly, in small groups, or occasionally in small colonies. Frequently the bacilli are not enclosed within cells, but they are also commonly found in small groups within detached endothelial cells; they are rarely found within circulating leucocytes.

Reaction of the Tissue Cells.

Leucocytes and Small Lymphocytes.

Rabbit 1092.—In the lungs bacilli are very rare, but both where these occur and throughout the rest of the tissue leucocytes and small lymphocytes are generally distributed in fairly large numbers. In both the liver and the kidney caseous foci are present, bacilli being rare in the former organ but fairly numerous in the latter; in both organs there is a general infiltration of lymphocytes and leucocytes and large numbers of these cells surround the lesions.

Rabbit 956.—The liver contains many lesions which are commencing to caseate, and contain few bacilli; in the interior of these foci leucocytes and lymphocytes are present in fairly large numbers. The lesions found in the kidney are in the same condition.

Rabbit 326.—The lungs are congested and contain numerous small consolidated foci in which bacilli are scanty; within the lesions and also in the rest of the tissue leucocytes and small lymphocytes are numerous; a few of the leucocytes contain bacilli. The liver contains many small lesions which are invaded with lymphocytes and leucocytes and contain few bacilli; the lymphocytes are in excess of the leucocytes. The lesions in the kidneys are rather more extensively caseous than in the liver and contain more bacilli; at the periphery of the lesions lymphocytes are abundant and leucocytes are relatively scanty. A mesenteric gland contains very large numbers of bacilli and is extensively caseous; there are many broken down leucocytes in the lesions but leucocytes which retain their oxyphil protoplasm are not numerous.

Rabbit 957.—The lungs contain many caseating foci in most of which bacilli are scanty; the lesions contain numerous leucocytes. In the liver bacilli are rare, but there are several minute lesions in which lymphocytes are much in excess of leucocytes.

Rabbit 294.—In the lungs bacilli are scattered throughout the tissue, and are abundant in some of the consolidated patches; leucocytes are distributed generally in large numbers, but show no close association with the bacilli. Bacilli are disseminated throughout the liver in large numbers, and have grown everywhere into colonies with the characteristic rosette formation; leucocytes are also very numerous throughout the specimen, and sometimes contain bacilli; small lymphocytes are less common than the leucocytes.

Rabbit 292.—The lungs are in a semi solid condition; throughout their substance bacilli are thickly scattered, and have grown into colonies; leucocytes are very abundant, and sometimes contain bacilli; small lymphocytes are also numerous. The liver is crowded with dense masses of bacilli; oxyphil-leucocytes are scattered about the tissue, but not in very large numbers; they are most numerous where caseous change is commencing; a few of the leucocytes contain bacilli; lymphocytes are slightly more numerous than the leucocytes. In the kidneys there are numerous small groups of bacilli, which occur sometimes in glomeruli and sometimes in the interstitial tissue; surrounding the bacilli there are aggregations of leucocytes and lymphocytes; the leucocytes are plentiful, and a few of them contain bacilli. The spleen is crowded with groups of bacilli, and shows a marked leucocytosis; many of the leucocytes contain bacilli. In a mesenteric gland, where also bacilli are very abundant, there is an aggregation of oxyphil leucocytes external to the colonies of bacilli.

Rabbit 1183.—In the lungs there is a general distribution of bacilli in moderate numbers, and leucocytes are plentiful, but are not specially attracted to the early tubercles to which the bacilli have given rise. The liver is crowded with dense masses of bacilli which have produced relatively little tissue destruction; lymphocytes and leucocytes are present in the lesions, the former being in excess of the latter; the leucocytes are not very numerous, and relatively few of them contain bacilli.

Rabbit 775.—In the lungs bacilli are numerous, but are rarely found within leucocytes; these cells are not very plentiful. The liver is crowded with dense masses of bacilli; leucocytes are not numerous, but some of them contain bacilli.

Rabbit 880.—In the lungs leucocytes are rather scanty, but a few occur both in the caseous nodules and in the rest of the tissue; small lymphocytes are rather more numerous. In the lesions found in the liver small lymphocytes are numerous, but leucocytes are scanty. The kidneys contain caseous and slightly caseous lesions in which leucocytes are very numerous.

Plasma Cells.

These cells have not been found in very large numbers in the lungs. They occur at the periphery of some of the caseating lesions but are not usually numerous and in some specimens none have been found. Collections of these cells have not been observed at the periphery of bronchioles or blood-vessels. The cells found which react to Pappenheim's stain are usually of more or less spherical outline, and not compressed or elongated. Plasma cells occur in the interlobular tissue of some of the severely affected livers but are not a conspicuous feature within or at the periphery of the lesions. Similarly in the kidneys these cells are not numerous in association with the tubercles, but occur, though not generally in large numbers, in the specimens showing patches of early cellular infiltration. On the whole, it seems that the avian bacillus does not stimulate a large production of plasma cells in the rabbit.

Endothelial Cells.

In none of the specimens showing advanced lesions is there any indication that the avian bacillus stimulates the endothelial cells to proliferate by mitotic division, and there is nowhere any tendency to the formation of new capillaries. In lesions which have become definitely caseous the pre-existing capillaries are obliterated; and in earlier lesions, breaks of continuity sometimes occur in the capillary walls, notably in the liver, the result being a small extravasation of blood, frequently accompanied by a small deposit of fibrin. The endothelial cells are capable of ingesting bacilli and may become detached from the vessel wall; these cells, containing one or more bacilli, are frequently seen amongst the free contents of the larger vessels.

In lesions found in the lungs the endothelial cells are often swollen and show a tendency to increase of their nuclei by direct division, but this increase is not sufficiently pronounced to lead to large accumulations of nuclei. In the liver, except when obliterated by caseation, the endothelial cells appear to be remarkably indifferent to the presence even of large numbers of bacilli; they may remain unaltered or they may become swollen, but, in these advanced stages of infection, they show no signs of active increase, and rarely signs of amitotic nuclear division or of fusion with adjacent parenchymatous cells. In the kidneys very little active change has been found in the endothelial cells. In lymphatic glands and spleens these cells sometimes show a slight tendency to nuclear division by amitosis and give rise to small nuclear accumulations resembling giant cells; but this tendency does not constitute a prominent feature of the lesions.

Epithelial Cells.

When consolidated foci are formed, the lung epithelium swells up, and the nuclei of the cells sometimes divide amitotically, but these divisions are not abundant. The cells of the pulmonary epithelium often contain bacilli.

The liver cells are the favourite site for the multiplication of bacilli, which often grow into a colony within the interior of a cell. Sometimes the nucleus is thrust to one side, and sometimes the bacilli form a ring with the nucleus in the centre. The nuclei of liver cells which contain bacilli show a tendency, often very strongly marked, to divide amitotically; the group of nuclei thus formed is a conspicuous object, particularly when surrounded by a complete circle of bacilli.

No definitely active change has been observed in the renal epithelium.

Fibroid and Caseous Changes.

Though caseous foci have been noted in a great many of the chronic lesions, the amount of caseous degeneration, relatively to the enormous number of bacilli present in some tissues, is remarkably small in acute infections. This is particularly the case with the liver, where the whole tissue may be crowded with colonies of bacilli without showing any completely necrotic or caseous foci. The lymphatic glands appear to be the organs which give way to caseous degeneration most readily. In completely caseous areas the bacilli are generally scattered about irregularly and do not exhibit the characteristic rosette formation.

The amount of fibroid change is, on the whole, small. Fibroblasts are found at the periphery of caseous lesions, but even in the milder infections these are not walled in by a broad fibrous zone. And in the majority of the lesions which contain numerous bacilli and show little or no caseous change, fibroblasts are few or absent.

Summary.

In early stages after intravenous inoculation the lungs show irritant effects similar to those found after intravenous inoculation of mammalian bacilli. During the second week slightly caseous foci make their appearance and resemble those produced by mammalian bacilli of relatively low virulence. Leucocytes are in excess of the normal, but only a few are found to contain bacilli. The characteristic of the livers is that the bacilli rapidly make their way into the parenchymatous cells and there commence to grow. Their invasion is accompanied, particularly at the outset, by protoplasmic degeneration of the parenchymatous cells and swelling up of the surrounding endothelial cells, but this tissue reaction soon subsides and, whilst the bacilli continue to grow within the liver cells, at this stage little further change is produced in the tissue. In these early stages of invasion, the nuclei of both endothelial cells and liver cells, more particularly of the latter, show a tendency to increase by direct division. No lesions, but a few bacilli, have been found in the kidneys. Like the liver, the spleen is highly favourable to the growth of bacilli, which arrive in this organ in fairly large numbers during the first 24 hours and progressively increase from this time onwards. At the end of a fortnight numerous colonies and rosette formations are found. The amount of tissue damage is slight, but there are numerous minute areas of partial necrosis. The endothelial cells in affected areas swell up, their protoplasm degenerates, and their nuclei often increase by direct division.

During the first fortnight after subcutaneous inoculation there is very little microscopic evidence of dissemination, and the condition of the local lesions is very similar to that found after subcutaneous inoculation with mammalian bacilli.

In later stages of infection it is found that both after feeding and after subcutaneous inoculation the bacilli disseminate, but not readily and not in large numbers. But after intraperitoneal inoculation the dissemination is abundant. General dissemination and very profuse multiplication of bacilli is also the rule after intravenous inoculation, but when the dose inoculated is very small the bacilli tend to be localised in caseous nodules and their growth is much more restricted.

The histological features found in the later and in the fatally terminated cases may be divided broadly into two classes. There is the dissemination of chronic caseous tubercles, found after subcutaneous inoculation and after feeding; and there is the diffuse distribution with enormous multiplication of bacilli, within parenchymatous cells and not accompanied by tubercle formation, which is the characteristic of intravenous and intraperitoneal inoculations. The chronic lesions present no diagnostic difference from chronic lesions produced by mammalian bacilli. The distinctive features of the acute infections are the freedom with which bacilli circulate in the blood-stream, their very abundant multiplication within parenchymatous cells, the absence of tubercle formation, and the slight amount of tissue destruction.

C.—IN GUINEA-PIGS.

The specimens examined illustrate the relatively small amount of tissue damage which the avian bacillus, by subcutaneous or intraperitoneal inoculation, produces in the guinea-pig, as compared with mammalian bacilli, and the relative scarcity of bacilli found in the tissues.

These results appear to be characteristic of the reaction of the guinea-pig towards the avian bacillus and to be independent of the dose. Guinea-pig 1494, for example, was inoculated intraperitoneally with the whole of a culture; but 37 days afterwards the bacilli were scanty in the lung and were evidently in process of destruction; they were only present in small numbers in the liver and spleen, and none were found in the kidneys; in a mesenteric gland, however, they were fairly numerous. The bacilli had produced small patches of slight necrosis, which were infiltrated with leucocytes and lymphocytes, but there was no formation of lesions typical of mammalian tuberculosis.

The susceptibility of the various organs examined appears to be proportionate to that observed after infection with mammalian bacilli, though less in degree; the kidneys are again conspicuous by their high powers of resistance.

These general results are put together in the following table:—

DISSEMINATION OF AVIAN BACILLI IN THE GUINEA-PIG.

Mode of Infection.	Duration of Experiments in Days.	Virus.	Dose.	Number of Animal.	Presence or Absence of Bacilli in Various Organs.			
					Lungs.	Liver.	Kidneys.	Other Organs Examined.
Subcutaneous	54	P II.	1 mg.	1532	Absent	Bacilli absent; many slightly caseous foci.	Absent	No bacilli or lesions found in the spleen.
Subcutaneous	109	A V.	Emulsion of pheasant's liver.	1962	Scanty	Bacilli absent; a few caseous foci.	Absent	Bacilli rather scanty in a portal gland.
Intraperitoneal	19	P II.	5 mg.	1533	Fairly numerous.	Fairly numerous.	Scanty	—
Intraperitoneal	37	P II.	1 glycerin-serum culture.	1494	Scanty; many fragments.	Rather scanty	Absent	Bacilli are scanty in the spleen but fairly numerous in a mesenteric gland.
Intraperitoneal	52	A VIII.	1 mg.	2503	Small groups	Generally distributed in small numbers.	Absent	Bacilli scanty in the spleen.
Intraperitoneal	57	A VI.	1 mg.	2624	Rare	Scanty	Absent	Bacilli are scanty in the spleen.
Intraperitoneal	180	A IV.	1 mg.	2131	Absent	Bacilli absent; some slightly caseous foci.	Absent	Bacilli absent in a mesenteric gland.

Summary.

After subcutaneous inoculation, and also after intraperitoneal inoculation, the guinea-pig is much less susceptible to the avian than to the mammalian tubercle bacillus. The amount of tissue damage produced by the avian bacillus is relatively slight, and in no instance have the bacilli increased in large numbers.

D.—IN OTHER MAMMALS.

CALVES.

After intravenous inoculation with a large dose of culture (10 mg. or more) or with a tissue emulsion rich in bacilli, rapidly fatal infection is produced in young calves. It is associated with general dissemination and abundant multiplication of bacilli; the bacilli often grow into solid clumps, and typical rosettes are also found. The severity of the infection is also shown by the abundant deposits of fibrin which frequently occur. The amount of tissue destruction is relatively small, and there is an absence of tubercle formation. These results offer an interesting resemblance to the condition found in young calves which have rapidly succumbed to the intravenous inoculation of strains of mammalian bacilli possessing low virulence for the bovine when inoculated subcutaneously. Points of difference are that with the avian bacillus the multiplication of bacilli is more profuse and, as indicated by the greater frequency of fibrinous deposits, the infection is rather more acute. In an exceptional case, Calf 566, the animal survived the intravenous inoculation of avian bacilli, and the bacilli disappeared from most of the organs.

But when a large dose (50 mg.) of the avian bacillus is inoculated subcutaneously, not more than a mild degree of general dissemination is produced, and in some cases the disease appears to have been almost limited to the nearest glands. The disseminated lesions are of a chronic, and generally of a definitely retrogressive, character. Caseation and giant cell formation are not uncommon, and the general structure of some of the lesions is indistinguishable from that of chronic tubercles produced by mammalian bacilli. Bacilli, when present in the lesions, are usually not numerous; but occasionally, *e.g.*, in the portal gland of Calf 412, the bacilli in some of the tubercles have multiplied abundantly.

MONKEYS

Like the calf, the monkey is highly susceptible to large doses (10 mg. and more) when inoculated intravenously, the effect usually proving fatal in less than a month. The amount of tissue damage is relatively small, and there is no formation of tubercles; but the bacilli multiply very abundantly, forming dense clumps and rosettes, particularly in the liver and spleen. In fact the general features of the infection resemble those produced in the rabbit by intravenous inoculation. As with the rabbit and the fowl, the liver and spleen of the monkey are more favourable sites than the lungs for the profuse multiplication of the avian bacillus. One monkey, inoculated intravenously with a smaller dose (1 mg.), died in 34 days but revealed only scanty deposits of bacilli.

To subcutaneous inoculation the monkey is highly resistant, and histological examination of most of the material provided has yielded negative results. In one exceptional case (Monkey 118), however, subcutaneous inoculation of 10 mg. was followed by acute disseminated tuberculosis identical with the disease produced by virulent mammalian bacilli. From the peculiar features presented by this case no definite conclusions can be drawn.

Feeding, even with a dose as large as 500 mg., failed to produce any infection.

SWINE.

Intravenous inoculation with 50 mg. and with a tissue emulsion rich in bacilli has caused fatal infection in 47 and 120 days respectively. The bacilli have disseminated everywhere in enormous numbers and grown into dense colonies and rosettes. They have caused relatively little tissue destruction, but giant cells are numerous.

After subcutaneous inoculation with 50 mg., no evidence of dissemination was found in one animal (Pig 264); but in another (Pig 180) there is a free distribution of bacilli throughout the tissues, and bacilli are found circulating in the blood-stream. Relatively little tissue destruction has been caused. The bacilli do not show signs of copious multiplication except in a mesenteric gland, where they are very numerous and form typical rosettes.

After intraperitoneal inoculation also the results are variable. One animal (Pig 104), after a dose of 10 mg. shows bacilli distributed in small numbers throughout the liver and a mesenteric gland; there are no organised tubercles, but numerous giant cells have been produced. Another animal (Pig 182), inoculated with the same dose, shows general dissemination and abundant multiplication of bacilli, with little tissue destruction, in all the organs examined except the kidneys.

Divergent results are also obtained after feeding. In two animals, fed with large doses, no disseminated bacilli have been found, though in one of them there are a few retrogressive tubercles in the lungs. In a third animal, fed with the same virus as one of the former but with 100 mg. instead of 50 mg., the general condition indicates a very free circulation of bacilli by the blood-stream. The bacilli have multiplied abundantly in all the tissues, but have caused very little tissue destruction and have not led to the formation of tubercles.

GOATS.

Intravenous inoculation with doses of 1 mg. and 10 mg. has produced rapidly fatal infection with general dissemination and abundant multiplication of bacilli but no tubercle formation and very little tissue damage.

After subcutaneous inoculation the results are irregular. In three animals doses of 10, 50, and 100 mg. have produced only slight evidence of dissemination; but in a fourth (Goat 48) 50 mg. has produced general dissemination and abundant multiplication of bacilli, with typical rosette formations. The lungs contain large, progressive, caseous lesions, but in the other organs the amount of tissue destruction is small and there are no tubercles. Enormous numbers of bacilli are found in the villi of the small intestine.

RATS.

The rat is highly resistant to subcutaneous inoculation, but the bacilli become disseminated and survive, though without active multiplication, for a very long period.

Intraperitoneal inoculation, even with the relatively small dose of 1 mg., is usually followed by general dissemination and abundant multiplication of bacilli, but without tubercle formation.

OTHER ANIMALS.

In a cat, 1 mg., inoculated intraperitoneally has produced general dissemination and enormous multiplication of bacilli, with very little tissue destruction.

The dog appears to be highly resistant both to subcutaneous and to intravenous inoculation. In one animal, subcutaneously inoculated with 25 mg., there are a few small, retrogressive lesions in the lungs and liver, but very few bacilli have survived. In another dog, the intravenous inoculation of 50 mg. produced remarkably little result. The bacilli have almost entirely disappeared, and have left behind only minute retrogressive lesions.

Mice react in the same way as rats after intraperitoneal inoculation, but are more susceptible to subcutaneous inoculation.

Ponies are highly resistant. Subcutaneously, 100 mg. has been followed by small deposits of bacilli in the spleen, but no other microscopic evidence of dissemination beyond the nearest gland; and after 50 mg. no microscopic evidence of dissemination has been found. After feeding with 600 mg., no microscopic evidence of dissemination beyond the glands draining the alimentary tract has been found. After 10 mg. intravenously, an animal shows general dissemination of bacilli in small numbers, but no evidence of progressive disease.

Summary.

After intravenous inoculation, calves, monkeys, swine, and goats are highly susceptible to the avian bacillus, which multiplies abundantly throughout the tissues, circulates freely in the blood-stream, and produces rapidly fatal infection without tubercle formation.

Calves are highly resistant to subcutaneous inoculation, but occasional disseminated tubercles of chronic type are found. No disseminated tubercles have been found in monkeys after feeding, and only in one case after subcutaneous inoculation. In swine variable results have been found after feeding, after subcutaneous inoculation, and after intraperitoneal inoculation; in some instances the animals have evinced high resistance, but in others the infection has resembled that produced by intravenous inoculation. In goats, subcutaneous inoculation is in the majority of cases followed by only slight evidence of dissemination, but in one animal severe progressive disease has been produced. The tissues of ponies infected subcutaneously, by feeding and by intravenous inoculation, exhibit very little evidence of dissemination.

E.—THE ACTION OF THE MAMMALIAN LEUCOCYTE UPON THE AVIAN BACILLUS.

In order to obtain some numerical estimates for the purpose of controlling my statements about the relations of leucocytes to the avian bacillus, a series of counts has been made, by the same method which I adopted for tissues infected with the mammalian bacillus.

81 counts were made, viz., 18 in tissues of calves intravenously inoculated, 42 in rabbits, and 21 in swine. Some observations have also been made on goats.

The general results of the counts indicate that leucocytes do not play an important part in attacking the avian bacillus. The percentage of leucocytes containing bacilli is low, except in some cases where the bacilli are present in overwhelming numbers and have made their way into every sort of cell capable of ingesting them.

In the tissues of intravenously inoculated calves, taking only the cases where definite estimates could be made, it was found that in an area containing 13,000 leucocytes 738 bacilli were present within leucocytes, the percentage of leucocytes containing bacilli being 2·5. This result may be compared with the counts made on calves intravenously inoculated with mammalian bacilli, where it was found that in a area containing 27,000 leucocytes 695 bacilli were present within leucocytes, the percentage of leucocytes containing bacilli being 1·5.

It has already been remarked that the swine's leucocytes ingest mammalian bacilli rather more frequently than the leucocytes of the bovine or rabbit. The same seems to be true for the avian bacillus, since I have found that in an area containing 16,000 leucocytes the percentage of leucocytes containing bacilli was 12·4. But too much weight must not be attached to this high percentage; it has been produced by the inclusion of counts where the whole of the tissue was swarming with bacilli, a condition not found in any of the swine infected with mammalian bacilli.

In the rabbit a comparison may be made between the action of the leucocytes on the avian and on the mammalian bacillus during the first fortnight after intravenous inoculation with 1 mg. of culture. The counts after avian infection show that in an area containing 17,000 leucocytes 10,431 bacilli were found; 221 of these were within leucocytes, the percentage of leucocytes containing bacilli being ·7, and the percentage of bacill within leucocytes 2·5. The counts after mammalian infection show that in an area containing 37,000 leucocytes 8,133 bacilli were found; 275 of these were within leucocytes, the percentage of leucocytes containing bacilli being ·7, and the percentage of bacilli within leucocytes 3·4.

In a series of counts on the tissues of rabbits in later stages of infection with the avian bacillus, after various modes of inoculation, the result was that in an area containing 18,000 leucocytes 335 bacilli were found within leucocytes, the percentage of leucocytes containing bacilli being 1. This may be compared with a series of counts on the tissues of rabbits in later stages of infection with mammalian bacilli, after intravenous inoculation, where the result was that in an area containing 17,000 leucocytes 350 bacilli were found within leucocytes, the percentage of leucocytes containing bacilli being 1·4.

In counts made on the tissues of goats infected with mammalian bacilli, the number of bacilli found within leucocytes proved to be very small; and in the specimens of goats' tissues infected with avian bacilli I have also found that the percentage of leucocytes containing bacilli is low.

The general conclusion appears to be that, in the animals mentioned, the leucocytes behave towards the avian bacillus very much in the same way as they behave towards the mammalian bacillus.

V.—COMPARISON OF RESULTS.

A.—Bacteriological.

When grown on the ordinary test media, the differences between the slimy growths of the avian tubercle bacillus and the dry growths of the mammalian are so striking that there can be no hesitation in pronouncing the organisms to be bacteriologically different. And it is often easy to distinguish between the two by the appearance of a film made from a culture, owing to the ready way in which the avian bacilli can be distributed uniformly over the cover-slip, whereas the much more sticky mammalian bacilli tend to adhere together in clumps.

Morphologically the avian bacillus exhibits more pleomorphism than the mammalian, and more frequently assumes true branching. When growing freely in the tissues the avian bacilli are generally very short and are often distinguishable from mammalian bacilli by their assumption of a rosette form. But when growing scantily and under difficulties the avian bacillus is longer and often stains irregularly; under these conditions it is indistinguishable, morphologically, from the mammalian bacillus.

B.—Histological.

The two most striking differences between the action of avian and mammalian bacilli upon the tissues are:—(1) in fowls, after intravenous inoculation, the avian bacillus multiplies very abundantly, whereas the mammalian bacillus either dies out completely or only grows with difficulty and in scanty numbers; (2) in guinea-pigs, after subcutaneous or intraperitoneal inoculation, the avian bacillus usually fails to grow, whereas the mammalian grows readily and produces disseminated disease.

The above differences are enough to make it clear that the effects produced by the two varieties of bacilli are different, not merely in degree, but in kind.

The special feature of the rabbit is that it is readily susceptible to both varieties of bacilli. There is, however, a difference to be found in the acute infections. Compared with the mammalian, the avian bacillus grows more abundantly, produces less tissue destruction, and causes much less inflammatory reaction at the margin of the lesions.

With regard to the majority of other mammals, the tissues of which I have investigated, the general results may be summarised by stating that after intravenous inoculation, alike with avian and with mammalian bacilli, an adequate dose produces highly acute disease, which is not typical of tuberculosis; after other methods of infection the avian bacillus has rarely produced progressive or disseminated disease, and, with only a few exceptions, has not succeeded in multiplying in the tissues; lesions of a chronic type, produced by the avian bacillus, have, however, sometimes been found, and some of these are histologically indistinguishable from lesions produced by mammalian bacilli.

Proceeding to further details in which my histological investigations bring to light specific and non-specific resemblances or differences between the effects of avian and those of mammalian bacilli, it is of interest to consider the manner in which the bacilli in question may kill their animal host.

Taking first the non-specific irritant effects of the avian bacillus, it is to be noted that in young calves which have died after intravenous inoculation with avian bacilli the acute inflammatory condition of the lungs is sufficient to account for death. In this connection it must be borne in mind not only that irritant effects on the lungs are inseparable from this mode of inoculation but also that the pulmonary tissue of the young calf is extremely susceptible to inflammatory processes. Comparison with other animals shows that the production of death through this irritant effect on the lungs is exceptionally common in the calf. In fowls, which have died as the result of intravenous inoculation of avian bacilli, the lungs show very little tissue damage as compared with other organs. In rabbits the lungs are certainly damaged by intravenous inoculation; in the early stages there is congestion, and in the terminal stage both congestion and patches of consolidation are found; but the incidence of the disease falls with greatest severity on other organs, notably the liver and spleen. In monkeys also the liver and spleen are the organs which suffer most severely after intravenous inoculation, whilst the amount of tissue damage in the lungs may be relatively slight. For example, in my report on the lungs of Monkey 198, which died 28 days after intravenous inoculation with 100 mg. of culture, I wrote:—"There are no patches of consolidation or tissue destruction: the majority of the alveolar walls are not even thickened, but appear perfectly normal in structure, though they contain large numbers of bacilli. The absence of irritant action on the part of the bacilli is remarkable." And regarding the lungs of Pigs 236 and 248, which died after intravenous inoculation, I wrote, after referring to patches of thickening and consolidation in these lungs:—"The bacilli are not confined to these thickened patches, but are growing freely amongst otherwise normal alveolar walls, the total area of which is largely in excess of the consolidated tissue." In Goats 56 and 58, which died after intravenous inoculation, a good deal of congestion was observed in the lungs.

It appears from the foregoing considerations, that, when artificially introduced into the circulation, the avian bacillus may kill its host by the production of an acute inflammatory condition of the lungs but that, with the exception of young calves, this is not the usual way in which most species of animals succumb to the effects of the avian bacillus even when intravenously inoculated. Turning to organs other than the lungs, there is no evidence, in any of the specimens which I have examined, that an acute inflammatory condition has been produced.

Next there is a class of cases, where fatal infection has been produced, which are characterised by an enormous multiplication of avian bacilli, associated with relatively little tissue destruction. In most of these the bacilli have been introduced directly into the blood-stream, but in some, though the histological evidence, post mortem points to free circulation in the blood-stream, some other mode of infection had been adopted. In these cases the most obvious explanation is that the profuse growth of bacilli at the expense of the tissue cells of such organs as the liver and spleen produced death by the impairment of function of vital organs. How far the action of a bacterial toxin may also be postulated is less clear. Histological examination proves that there is not the typical breaking down and caseation of the tissues which is associated with the specific toxicity of the tubercle bacillus: and I do not know of any evidence that some other generally diffusible toxin is produced. The production of death by a profuse multiplication of avian bacilli is the common result, after intravenous inoculation, in the fowl, the rabbit, and the monkey, and is also met with in swine and goats. I have also observed it after intraperitoneal inoculation in the fowl, rabbit, and cat; after feeding, in the case of Pig 178, and after intraperitoneal inoculation, in the case of Pig 182, a similar profuse multiplication of bacilli was found, though these animals were killed when in apparently good health. It may also be mentioned that in rats, after intraperitoneal inoculation, and in mice, after either intraperitoneal or subcutaneous inoculation, there is an abundant intracellular growth of bacilli which may persist for a long time without causing the death of the animal. But, these exceptional cases notwithstanding, it must be recognised that one of the typical modes in which the avian bacillus produces death is by the profuse growth of the bacilli in vital organs.

The third and most important mode in which the avian bacillus produces death is by a slow and steadily maintained toxic action upon the tissues. This is well seen in the fowl both after experimental feeding, where the disease resembles that found in naturally occurring infection, and after subcutaneous inoculation. It also occurs in the rabbit, particularly after subcutaneous inoculation, and, less frequently, after experimental feeding. The characteristic of this type of fatal infection is the local conflict between the bacilli and the areas of tissue which contain them. There is a progressive growth and continuous destruction of bacilli associated with a progressive expansion of caseous foci. The bacilli grow under difficulties, and, though eventually they may completely gain the upper hand in some of the lesions and then grow profusely, they are more usually scanty; and it appears probable that the material liberated by the constant breaking down of bacilli is the cause of the toxic effects which are manifested histologically in the production of extending caseous tubercles. Similarly, a toxic action due to the liberation of material from disintegrated bacilli is also probably the cause of death in the guinea-pig. This animal is an unfavourable medium for the multiplication of the avian bacillus, but not unfrequently dies within a few weeks after inoculation; and in some instances the evidence of necrotic change, particularly in the liver, points to a toxic effect which may well be the product of an abundant destruction of bacilli.

This third mode in which the avian bacillus kills its host is closely comparable to the common manner in which the mammalian bacillus produces death. The way in which the avian bacillus kills a calf after intravenous inoculation is also very similar to what is produced in similar experiments with mammalian bacilli upon calves. But though there are interesting parallels in rodents, and occasionally in other mammals, it is not typical of the mammalian bacillus to overwhelm the tissues with a profusion of intracellular growth such as is commonly produced by the avian bacillus in experimental infections.

The final pathological effects of the avian bacillus on the animal body have been dealt with first as being the most important; but it is also interesting to consider the intermediate stages. In order to kill an animal the avian bacillus must first disseminate in the vascular system, and then it must escape from this system and attack the adjacent tissues. How does it circumvent the endothelial cell?

It appears probable that the avian bacillus often passes directly through an endothelial cell or between contiguous endothelial cells without detention therein. In no other way is it possible to explain the frequent appearance of bacilli within parenchymatous cells at very early periods after intravenous inoculation. At the same time there is clear evidence, in these early stages of infection, that the endothelial lining forms at least a temporary barrier against the progress of many of the bacilli. In order to make good their escape, these bacilli must multiply, and this they proceed to do within the endothelial protoplasm. Influenced by the presence of the bacilli, the endothelial cell tends to swell up, and direct division, or partial division, of its nucleus often occurs; these changes retard the progress of the bacilli. What follows depends upon the endothelial cell's capacity for resistance. When the bacilli are present in large numbers in the blood-stream, this resistance is rapidly overcome, and the bacilli, escaping through this barrier, commence to grow profusely in the tissues. But when only a few bacilli are present in the blood-stream, the endothelial element, in cases where it is not completely successful, offers a prolonged resistance, and the chronic, caseous type of tubercle is the result. These two widely different results may be harmonised by assuming that the irritant or stimulant action of the avian bacillus upon the endothelial cell is relatively feeble, and hence only continues to play a conspicuous histological part in the milder types of infection. And one of the essential differences between the avian and the mammalian bacillus is that the latter exerts a more powerful irritant or stimulant action upon the endothelial cell, with the histological consequence that there is much less tendency for the lesions to assume one or other of two widely different types. The endothelial resistance is sufficiently vigorous both to prevent a profuse invasion of bacilli into the tissues and to produce a more exuberant type of tubercle than the characteristic chronic lesion caused by the avian bacillus.

When the avian tubercle bacillus has penetrated the endothelial lining and arrived in the adjacent tissue, it may either (1) grow abundantly, with relatively little tissue damage, or (2) grow slowly and with difficulty, and produce lesions which are centrally caseous and are completely circumscribed by fibrous tissue. The former type of growth is found where bacilli are circulating freely in the blood-stream, and the latter where the bacilli have not invaded the blood-stream in large numbers. Common to both types of lesion there is (*a*) a tendency to the protoplasmic fusion of individual cells, and (*b*) a tendency for the cell nuclei to multiply by direct division. In (1), (*a*) is at first limited, because the bacilli find intracellular growth so favourable that they do not, for a time, overstep the limits of individual cells; and this freedom of growth also limits (*b*), because individual nuclei become closely wrapped round with a cluster of bacilli and have no room to multiply. In (2), (*a*) sets in early and soon progresses to a focus of complete necrosis, whilst (*b*) is limited to the periphery of the necrotic area and may there occur profusely.

Comparing the action of the avian with that of the mammalian bacillus, it is noteworthy that lesions produced by the latter do not usually fall into one or other of the widely different types, (1) and (2), but are more frequently of an intermediate character, exhibiting less profuse intracellular growth than (1) and a tendency to stimulate the tissue to more proliferative change than is found in (2). I have found no fundamental difference in the relations of the wandering cells to the lesions produced respectively by the avian and the mammalian bacillus.

VIRUSES INVESTIGATED.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
B IV.	Baboon :— 76 Chimpanzee :— 24 Dog :— 116 Fowls :— 414 420	Chimpanzee 22. Dog 116. Dog 150. G.P. 2528 from Dog 150. Rabbit 1899. Fowl 394.
B VII.	—	Fowls :— 442 444
B VIII.	—	Dogs :— 142 156
B IX.	Dog :— 130 Fowl :— 492	Chimpanzee 18. Dog 130. Dog 136. G.P. 2935 from Dog 136. Fowl 430. Fowl 432. Monkey 126. Monkey 152.
B X.	Pig :— 138	
B XI.	Parrots :— 14 16 Ponies :— 12 30	
B XII.	Dog :— 160 Pony :— 6	Dog 160. Pony 6.
B XXII.	—	Dog 126.
B XXVI.	Bovines :— 414 416 452 466 474 476 923 925 1017 1023 1025 1027 1033 1035 1039 1043 Fowls :— 80 82 338 412 458	Fowl 84. Fowl 450. Fowl 516. G.P. 3331. Parrot 6 Parrot 8. Rabbit 1903. Rabbit 1904. Rabbit 1908.
B XXVI.—*cont.*	Parrots :— 6 8 Rabbit :— 488	
B XXIX.	Bovine :— 438	
B XXX.	Bovine :— 438	
H 8. S.C.	Bovines :— 923 925 1017 1023 1025 1027 1033 1035 1039 1043 Monkeys :— 67 69 71	
H 13. A.D.	Bovines :— 1175 1177 1203 1207 1237 1327	Calf 1175. Calf 1177. Calf 1237.
H 49. T.C.	Bovines :— 1213 1263 1347 1501 Rabbit :— 1181	
H 50. P.H.	Pigs :— 49 55	
H 53. D H.	Bovines :— 1135 1391 1435 1477 1507 1535 1545 Fowls :— 19 21 Goat :— 65	Human lupus (*b*) Calf 1155. Calf 1199. Calf 1257. Calf 1295. Calf 1333. Pig 85.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
H 53. D.H.—*cont.*	Monkeys :— 93 101 103 109 135 Pigs :— 89 121	
H 54. C.W.	Bovines :— 923 925	
H 56. F.T.	Bovines :— 1033 1035 Pigs :— 37 43	
H 59. L.B.	Chimpanzee :— 5	
H 60. W.B.	Bovines :— 989 1081 1099 1107 1109 1425 Monkeys :— 81 83	Human brain. Human bronchial gland. Human lung. Human mesenteric gland. C. 1107. Rabbit 1228.
H 61. E.C.	Monkey :— 77	
H 67. E.P.	Pigs :— 125 127	Human scapular abscess. Calf 1143. Calf 1329.
H 68. R.B.	Bovines :— 77 1161 Fowls :— 15 16 Guinea-Pig :— 1988 Pig :— 75	Human sacral abscess. Calf 1167. Calf 1361. Cow 77.
H 69. F.K.	Bovines :— 1249 1267	Human meninges. Human mesenteric gland.
H 70. N.W.	Cats :— 25 27 29 41 Guinea-pig :— 1976	Human bronchial gland. Human cervical gland. Human lung. Human mesenteric gland.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
H 71. L.V.	Cat :— 43 Guinea-Pig :— 1989 Monkey :— 195	Human lupus (*a*). Human lupus (*b*).
H 72. H.C.	Bovine :— 1365	Human abscess of rib.
H 74. D.S.	Monkeys :— 85 89 91 Rabbit :— 1195	Human bronchial gland. Human mesenteric gland. Human spleen.
H 75. A.H.	Rabbit :— 1205	Human lung. Human peritoneal fluid.
H 76. G.M.	—	Human knee joint.
H 77. R.L.	Bovine :— 1349 Rabbit :— 1246	Human liver. Human mesenteric gland.
H 78. O.D.	Rabbits :— 1212 1215	Human bronchial gland. Human lung. Human meninges.
H 79. J.N.	Bovine :— 1243 Dogs :— 31 33 Rabbits :— 1175 1748	Human lung. Calf 1227. Calf 1243. Calf 1297. Calf 1305. Calf 1321. Calf 1325. Dog 31. Dog 33. Rabbit 1085.
H 80. D.M.	Guinea-Pig :— 2976	Human lung. Calf 1245. Calf 1265. Calf 1279. Calf 1281. Calf 1369. Calf 1375. Dog 19.
H 81. P.W.	Chimpanzee :— 7 Bovine :— 61 Dog :— 43 Fowl :— 131 Guinea-Pigs :— 2340 2454 Horse :— 1 Monkeys :— 95 97 99 Parrots :— 1 3 5 7 9 11	Human lung. Calf 1129. Calf 1145. Calf 1357. Calf 1399. Horse 1. Parrot 7.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
H 82. G.F.	Guinea-Pig :— 2444	Human lung. Human mesenteric gland.
H 83. G.C.	Bovine :— 61	Human bronchial gland. Human meninges. Human mesenteric gland.
H 84. M.S.	Monkeys :— 143 231	Human lupus.
H 85. H.B.	Bovines :— 1289 1373 Chimpanzee :— 11 Goat :— 63 Guinea-Pig :— 2575 Horse :— 11 Monkeys :— 119 121 123 125 153 Pig :— 117 Rabbit :— 1496	Human lupus. Chimpanzee 11. Monkey 121.
H 86. C.P.	—	Human meninges.
H 87. W.D.	Rabbit :— 1342	Human suprarenal capsule.
H 88. E.L.	Bovine :— 1271	Human mesenteric gland.
H 89. W.I.	Bovines :— 1275 1351	Human mesenteric gland. Human lung.
H 90. I.P.	Bovines :— 1383 1471 1489 1493 (Rabbit Series, intravenous)	Human retro-peritoneal gland. Human mesenteric gland Calf 1383. Calf 1471.
H 91. H.S.	Monkey :— 133	Human lupus.
H 92. D.N.	Bovine :— 1317 Horse :— 7 Monkey :— 177	Human lupus.
H 93. C.D.	—	Human lung. Human acne spot.
H 98. B.R.	Goat :— 69	Human cerebro-spinal fluid.
H 99. L.K.	Monkey :— 151	Human lupus.
H 100. R.S.	Bovines :— 1409 1419 1511 1517 1523 1543 Monkey :— 163	Human lupus.
H 101. E.G.	Monkey :— 211	Human lupus.
H 102. N.H.	Monkey :— 213	Human lupus.
H 103. N.S.	Monkeys :— 171 217	Human lupus.
H 104. E.R.	—	Human lung.
H 105. G.S.	Bovines :— 1405 1449 1521 1527	Human lupus.
H 106. K.R.	—	Human lupus.
H 107. H.H.	Bovines :— 1453 1497 1505 1513 1519 1549 Fowl :— 143 Monkey :— 251	Human lupus.
H 108. H.R.	Bovines :— 1417 1421 1509 1539 Monkey :— 165	Human lupus.
H 109. M.W.	Monkeys :— 185 197	Human lupus.
H 110 J.B.	Bovines :— 1483 1491 1515 Goat :— 79 Monkeys :— 179 181 183 Pigs :— 141 143	Human lupus (*a*). Human lupus (*b*).
H 111. S.E.	Bovines :— 1429 1459 Monkey :— 189	Human lupus.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
H 112. B.B.	Monkey :— 201	Human lupus.
H 114. A.U.	Monkeys :— 223 225	Human lupus.
H 115. N.G.	Fowl :— 133 Goat :— 81	Human cerebro-spinal fluid.
H 118. F.C.	—	Human sputum.
H 119. E.S.	—	Human sputum.
H 120. B.W.	—	Human sputum.
H 121. E.A.	—	Human sputum.
H 122. F.P.	—	Human sputum.
H 123. W.W.	—	Human sputum.
H 124. M.M.	—	Human sputum.
H 125. A.P.	—	Human sputum.
H 126. A.W	—	Human sputum.
H 127. R.R.	Calf :— 1585	Human sputum.
H 128. D.D.	Calves :— 1567 1579	Human sputum.
H 129. T.M.	—	Human sputum.
H 130. N.B.	—	Human sputum.
H 131. M.H.	—	Human sputum.
H 132. O.O.	—	Human sputum.
H 133. P.H.	—	Human sputum.
H 134. K.K.	—	Human sputum.
H 135. E.E.	—	Human sputum.
H 136. X.B.	—	Human sputum.
H 137. O.B.	—	Human sputum.
H 138. C.C.	—	Human sputum.
H 139. K.H.	—	Human sputum.
H 140. J.J.	—	Human sputum.
H 141. C.H.	—	Human sputum.
H 142. Q.H.	—	Human sputum.
H 143. L.L.	—	Human sputum.
H 144. D.S.	—	Human sputum.
H 145. G.W.	—	Human sputum.
P I. ...	—	Original material.
P II. ...	Cat :— 62 Fowls :— 32 158 162 164 Guinea-Pigs :— 1494 1532 1533 Goat :— 36 Monkey :— 118 Pigs :— 104 110 Rabbits :— 292 294 326 1538 1539 1540 1541 Rat :— 85	Original material. Monkey 118.
P III. ...	Bovine :— 318 Dog :— 122 Fowls :— 36 100 Goats :— 56 58 Mouse :— 67 Pony :— 14 Rat :— 79	Original material. Calf 282.
P IV. ...	Bovines :— 312 314 356 358 426 Pigs :— 124 126 132 134 Rabbits :— 307 461 462 463 555 557	Original material Calf 312. Rabbit 556. Rabbit 557.
P V. ...	Bovine :— 336 Rat :— 76	Original material.
P VI. ...	Bovine :— 326	Original material.
P VII. ...	—	Original material.
P VIII. ...	—	Original material.
P IX. ...	Bovine :— 334	Original material.
P X. ...	—	Original material.
P XI. ...	Bovine :— 338	Original material.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
P XII. ...	Bovines :— 376 444 450	Original material.
P XIII. ...		Original material.
P XIV. ...	Bovines :— 446 448 532 534 536 538	Original material.
P XV. ...	—	Original material.
P XVI. ...	—	Original material.
P XVII. ...	—	Original material.
P XVIII. ...	—	Original material.
P XIX. ...	Rabbit :— 622	Original material.
P XX. ...	—	Original material.
P XXI. ...	Bovine :— 412 Fowls :— 26 28 148 152 Monkey :— 154 Rabbits :— 878 880 1092	Original material.
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P XLVI. ...	Pig :— 164 Rabbits :— 1047 1048	Original material.
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P LXI. ...	—	Original material.
P LXII. ...	—	Original material.
E I. ...	Bovines :— 436 522 528 Fowls :— 234 490 Pony :— 16 Rabbits :— 934 1309	Original material.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
E II.	Bovines :— 404 406 506 578 580 592 594 596 626 628 638 Guinea-Pigs :— 1977 1978 2213 2579 Lemurs :— 16 18 Monkeys :— 168 180 184 186 202 208 212 216 220 Pigs :— 184 186 Ponies :— 18 20 Rabbits :— 1082 1083 1331	Original material. Calf 404. Calf 504. Calf 506. Calf 590. Calf 596. Fowl 398.
E III. ...	Bovines :— 440 508 Fowl :— 154 Guinea-Pigs :— 2113 2115 2116 Lemur :— 14 Pig :— 194 Rabbit : 1232	Original material.
E IV. ...	Baboons :— 72 74 Bovines :— 410 498 502 582 586 602 614 620 630 Fowls :— 134 136	Original material. Baboon 72. Baboon 74. Calf 408. Calf 498. Calf 502. Calf 616. Fowl 438. Fowl 440. Monkey 178.
E IV.—*cont.*	Guinea-Pigs :— 1979 1980 2487 Monkeys :— 178 182 218 Pigs :— 188 190 Ponies :— 22 24 Rabbits :— 1080 1418 1699	
E V. ...	Rabbit :— 1445	Lung of horse. Spleen of horse.
Z I. ...	Rabbits :— 520 758	Lung of gnu.
Z III. ...	Bovine :— 510 Rabbits :— 1166 1172	Lung of antelope. Rabbit 1041. Rabbit 1241. Rabbit 1368.
Z VI. ...	—	Mesenteric gland of cat.
A I. ...	Fowl :— 146 Monkey :— 160 Rabbit :— 1090 Rat :— 102	Intestinal lesions of touracon.
A II. ...	Bovine :— 432 Fowls :— 92 96 Rat :— 122	Spleen of pigeon.
A III. ...	Goat :— 40	Lung of crane.
A IV. ...	Fowls :— 108 298 Guinea-Pig :— 2131 Mice :— 84 87 88 90 Rat :— 86	Liver of Pheasant I. Calf 456. Calf 494. Fowl 298.
A V. ...	Guinea-Pig :— 1962 Rabbits :— 956 957	Spleen of Pheasant II.
A VI. ...	Guinea-Pig :— 2624 Parrots :— 2 4	Spleen of Pheasant III.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
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A VIII. ...	Bovines :— 524 544 Guinea-Pig :— 2503 Pig :— 248 Pony :— 2 Rat :— 133	Spleen of Fowl B.
A X. ...	Bovines :— 546	Fowl D. Rabbit 1866.
A X.—*cont.*	Bovines :— 558 560 564 566 570 Dog :— 162 Fowls :— 264 392 Goats :— 42 48 Mouse :— 105 Monkeys :— 188 196 198 Parrot :— 20 Pigs :— 236 264 Pony :— 32 Rabbit :— 1791	

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1748	51, 52	
1791	184	
1866	—	272, 273
1899	—	212, 216
1903	—	216
1904	—	216
1908	—	216
Rats :—		
76	89	
79	201	
85	201	
86	201, 203	
102	202	
122	200, 203	
125	202	
127	203	
133	203	

COLOURED PLATES.

PLATE I.

STAIN.—Carbol-fuchsin followed by eosin and methylene blue.

MAGNIFICATION.—×600.

The figures on this plate illustrate the development of the tubercle in its early stages. Each figure represents a lesion produced in a liver of a rabbit after inoculation in the vein of an ear with 1 mg. of finely emulsified culture of mammalian tubercle bacilli.

Before describing the figures in detail, attention may be drawn to Fig. 9, which shows a typical tubercle, and to Figs. 1, 2, 5, 7, and 8, which show how such a lesion is produced.

Fig. 1. 24 hours after inoculation with B III. (a typical virus of bovine origin). In the right half of the figure two bacilli are seen in contact with the endothelial wall of a capillary; immediately to the left of these bacilli is the nucleus of an endothelial cell showing no pathological change. At the bottom of the figure is the wall of another capillary, showing three bacilli within an endothelial cell, the nucleus of which is slightly distorted; to its right is the more darkly staining nucleus of a small lymphocyte.

Fig. 2. 24 hours after inoculation with H 90. I.P. (a virus of human origin which in the series of experiments to which this rabbit belongs produced a much milder type of infection than the preceding bovine virus). A few bacilli are seen in contact with and partly within an endothelial cell. The nucleus of this cell is swollen and, on the left, a small fragment of it has split off.

Fig. 3. Three days after inoculation with H 90. I.P. A bacillus is seen definitely within a histologically normal liver cell.

Fig. 4. 24 hours after inoculation with B III. A spherical fragment of culture is found between two endothelial nuclei.

Fig. 5. 24 hours after inoculation with H 7. C.M. (a virus of human origin and of high virulence for the bovine and rabbit). This specimen illustrates more advanced change in the endothelium. The right wall of the capillary in the centre of the figure shows four distorted nuclei; a bacillus is found close to the lowest of these. Above and to the right of these four nuclei there is a nucleus showing an early stage of mitosis. Immediately above the bacillus there is some distorted and apparently dividing nuclear material which probably belongs to a parenchymatous cell. Noteworthy features of the lesion are (1) commencing fusion of endothelial and parenchymatous elements and (2) increase of nuclei belonging to these elements.

Fig. 6. 24 hours after inoculation with H 90. I.P. This illustrates the irritant action of the tubercle bacillus upon the endothelial cell. Following from left to right the vascular channel which passes across the field, one sees numerous endothelial nuclei of irregular outline and unequal amounts of chromatin. These nuclei are in process of increase by direct division. At the left end of the channel there are a few desquamated endothelial cells. At the right of the figure bacilli are seen within the protoplasm of slightly disintegrated liver cells. The lesion has been invaded by numerous small lymphocytes and by a few multinuclear leucocytes.

Fig. 7. Three days after inoculation with H 7. C.M. An early tubercle. In the centre of the lesion is the faint and distorted nucleus of a liver cell, surrounding which, above, below, and to the right, are nuclei of endothelial origin. The diffuse blue stain shows the escape of nuclear material outside the nuclear membrane. This diffuse staining is very characteristic of an early lesion. The nuclei are found in a matrix of protoplasm which is no longer marked off into individual cells. This protoplasm contains four bacilli. To the left of it are two nuclei of liver cells showing early change, viz., slight irregularity of contour and slight intensification of the nuclear stain. The two elongated nuclei at the bottom of the figure show early change in the endothelium. The figure also shows some small lymphocytes and one leucocyte.

Fig. 8. Eight days after inoculation with H 90. I.P. The relation of the endothelium of the capillary channels to early tubercle formation. The structure of the lesion is similar to that shown in the preceding specimen.

Fig. 9. Three days after inoculation with H 7. C.M. A typical tubercle, with a peripheral infiltration of small lymphocytes and finely granular oxyphil leucocytes. Some of the red dots in the centre of the lesion may be fragments of bacilli, others may be nucleoli of liver cells. Compare Figs. 7 and 8.

Figs. 10–14. Whereas Figs. 1–9 illustrate the essential structural processes of tubercle formation, Figs. 10–14 illustrate a concomitant factor which is often present and may obscure, or complicate, those essential processes. This factor is the attraction of polymorphonuclear leucocytes, owing to the irritant action of the bacillus and of the disintegration products attributable to the bacillus.

Fig. 10. Three days after inoculation with H 7. C.M. A small focus completely filled with leucocytes, one of which is dividing.

Fig. 11. From the same section as Fig. 10. A single leucocyte in process of amitotic division.

Fig. 12. 24 hours after inoculation with H 90. I.P. The splitting and multiplication of the endothelial nuclei is complicated by the invasion of leucocytes. There is a bacillus in the centre of the field but none are present within leucocytes.

Fig. 13. 24 hours after inoculation with B III. The condition is similar to that in Fig. 12, but has been produced by a more virulent virus. The thread-like material in the centre is fibrin. Immediately to the right of this is a bacillus. A short distance below this bacillus is a dividing leucocyte. To the right of this cell is a group of partially disintegrated leucocytes, one of which contains a bacillus. Above and slightly to the right of this group is a bacillus within a liver cell, the nucleus of which is splitting into two. Many small lymphocytes are associated with the leucocytes.

Fig. 14. Five days after inoculation with H 7. C.M. This is a typical early tubercle. Compared with Fig. 9, the lesion is less compact, there is more nuclear splitting, the diffuse escape of chromatin has cleared up, and caseation has not yet commenced. It is important to note that early lesions presenting the confused appearance of Fig. 14 are much commoner than the more easily analysed arrangement seen in Fig. 9. After studying the figures preceding it, it will be seen that in Fig. 14 the same processes are at work, viz., nuclear splitting of the endothelial and parenchymatous elements and infiltration with leucocytes and lymphocytes. It is specially important to note that the more advanced tubercle commonly originates from a focus such as Fig. 14, and not necessarily by expansion of a simpler lesion such as Fig. 9.

PLATE I

[illegible] and malignant [illegible]

[illegible]

[illegible] The [illegible] in a [illegible] of [illegible] which [illegible] individual cells. [illegible] the left of [illegible] showing early change, viz. [illegible] and slight condensation [illegible] The two elongated nuclei [illegible] early change [illegible] The figure also shows [illegible]

[illegible] days after inoculation with [illegible] the [illegible] [illegible] shown in the [illegible]

[illegible] days after inoculation with [illegible] with a [illegible] lymphocytes and [illegible] may be fragments of [illegible] liver cells. Compare [illegible]

[illegible] Whereas Figs. [illegible] illustrate [illegible] This factor is the [illegible] leucocytes, owing to the [illegible] bacillus and [illegible] the disintegration [illegible]

[illegible] days after inoculation with [illegible] completely filled with [illegible]

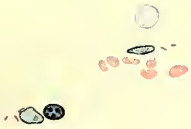
[illegible] from the same section as Fig. [illegible] of mitotic division.

[illegible] hours after inoculation with [illegible] and multiplication of the [illegible] by the invasion [illegible] a bacillus in the [illegible] leucocytes.

[illegible] hours after inoculation with [illegible] that in Fig. 12 but [illegible] a more virulent virus. The [illegible] A short [illegible] leucocyte. [illegible] a group of partially disintegrated [illegible] a bacillus [illegible] to the right of this group is a [illegible] which [illegible] lymphocytes [illegible]

[illegible] days after inoculation with [illegible] a typical early tubercle. Compared [illegible] is less compact [illegible] of [illegible] has not yet [illegible] that early [illegible] Fig. 14 are [illegible] easily analysed are [illegible] After studying the figures [illegible] in Fig. 13 the same [illegible] splitting of the [illegible] It is [illegible] advanced [illegible] Fig. [illegible]

PLATE I.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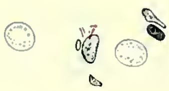
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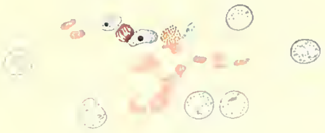
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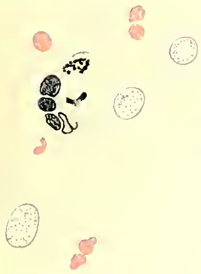
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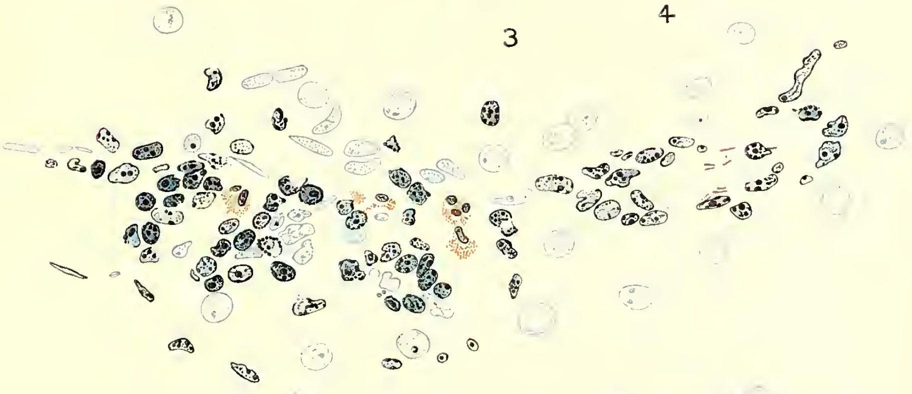
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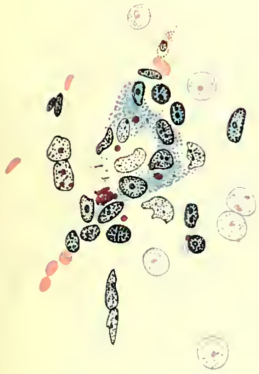
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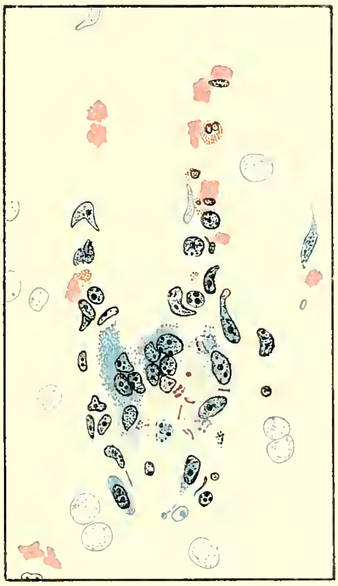
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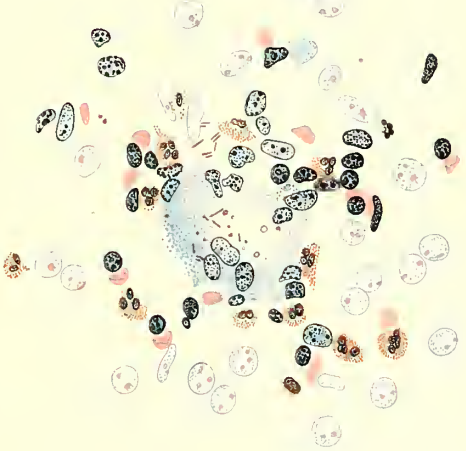
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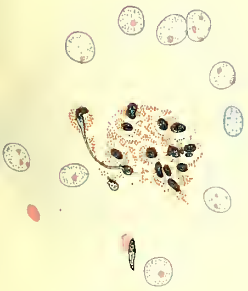
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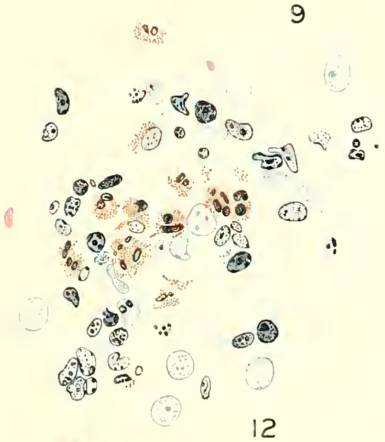
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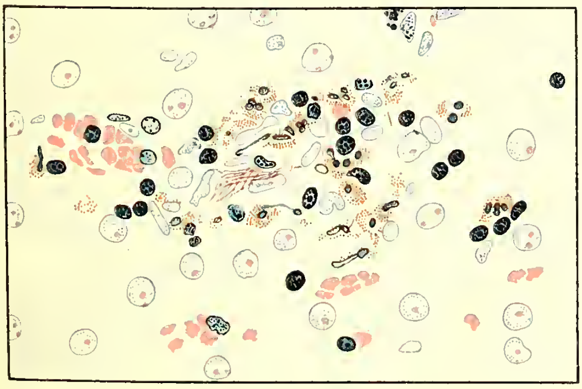
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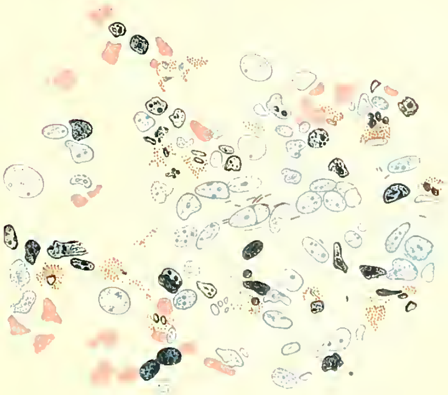
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J. R. Ford del.

Shiells & Ford, lith.

PLATE II.

STAIN.—Carbol-fuchsin followed by eosin and methylene blue.

Fig. 1. × 600. Liver of rabbit, eight days after intravenous inoculation in the ear with 1 mg. of culture of H 90. I.P. (a virus of human origin of comparatively low virulence for the series of experiments to which this animal belongs). The specimen illustrates an early stage of what will probably become a chronic or retrogressive type of tubercle, situated in the lower half of the field, towards the right. There is a small deposit of bacilli amongst partially disintegrated liver protoplasm. Surrounding this area, and apparently travelling towards it from Glisson's capsule, is an infiltration consisting mainly of small lymphocytes. There is also distortion and splitting of the nuclei of endothelial and liver cells. A nucleus in the upper part of the drawing, towards the right, shows a mitotic figure.

Fig. 2. × 470. Liver of rabbit, 10 days after intravenous inoculation in the ear with 1 mg. of culture of B III. (a typical virus of bovine origin). A tubercle of progressive type, at the stage when the fixed tissue cells are breaking down and, owing to partial fusion and degeneration of their protoplasm, are no longer distinguishable as individual cells. These changes in the protoplasm, being accompanied by shrinkage and vacuolation, have given rise to the appearance of a reticulum. Multiplication, by fission, of the nuclei of the fixed tissue cells is conspicuous ; and it is noticeable, particularly in the left half of the field, that bacilli are in immediate contact with such nuclei. In the lower part of the field, to the left, is a small caseous area which, taken in conjunction with the adjacent nuclei, may be termed a "giant cell." The reticulum in this neighbourhood presents the appearance of processes streaming out from the "giant cell." Bacilli are more numerous in the "giant cell" than elsewhere. There is some infiltration of small lymphocytes and multinuclear leucocytes. None of the leucocytes show evidence of phagocytosis.

Fig. 3. × 600. From the same liver as Fig. 2. A well marked example of the capacity of the tubercle bacillus to stimulate amitotic nuclear division.

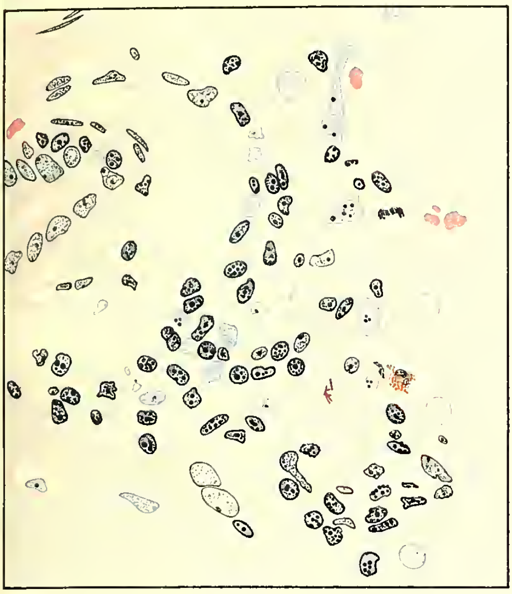
Fig. 4. × 600. Liver of Rabbit 1283, killed 29 days after inoculation in the vein of an ear with 50 mg. of killed culture of bovine bacilli. The section was studded with minute lesions like the two figured. In a few of the lesions one or two bacilli were demonstratable. The infiltration of the two "giant cells" by small lymphocytes is noteworthy. It serves to explain the fact that "giant cells" not uncommonly contain deeply staining, spherical nuclei which are identical with the nuclei of lymphocytes. Such nuclei do not indicate that "giant cells" are derived from lymphocytes ; they show that "giant cell" lesions, initiated in the fixed tissue cells, may be infiltrated by lymphocytes, and that the nuclei of the latter cells may survive therein. This is particularly the case in the milder types of infection, whether produced by dead or living bacilli. In the lesions of acute infections the lymphocytes are rapidly destroyed.

Fig 5. × 520. Lung of rabbit, 15 days after intravenous inoculation in the ear with 1 mg. of culture of B III. A portion of the wall of an artery showing a small tubercle which has developed from the endothelium and projects into the lumen of the vessel.

Fig. 6. × 275. Kidney of rabbit, 10 days after intravenous inoculation in the ear with 1 mg. of culture of H 7 C.M. (a human virus of high virulence for the bovine and rabbit). An early stage of acute infection. In the centre of the field are several bacilli, and in close association with these is some recently deposited fibrin, which has retained the eosin stain. Bacilli are also scattered about in the upper part of the figure ; some of them are close to, but not actually within, multinuclear leucocytes. The specimen as a whole illustrates the interstitial infiltration of leucocytes and small lymphocytes and the increased prominence of the connective tissue elements. These features are typical of early tubercle formation in the kidney. Here, as is usually the case, the lesion begins as an intertubular infiltration and does not arise from a glomerulus.

Fig. 7. × 294. Thoracic gland of Goat 1, which had been inoculated subcutaneously on four successive occasions with viruses of human origin, and died 34 days after the last inoculation. A peripheral lymph sinus, with a portion of the capsule in the upper part of the drawing, illustrating the passage of bacilli through the capsule into the sinus, and the presence, within the lumen of the sinus, of bacilli lying within detached endothelial cells.

PLATE II.



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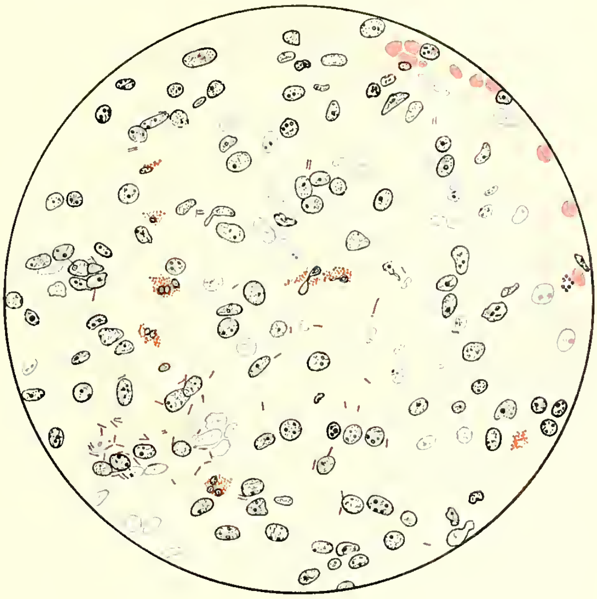

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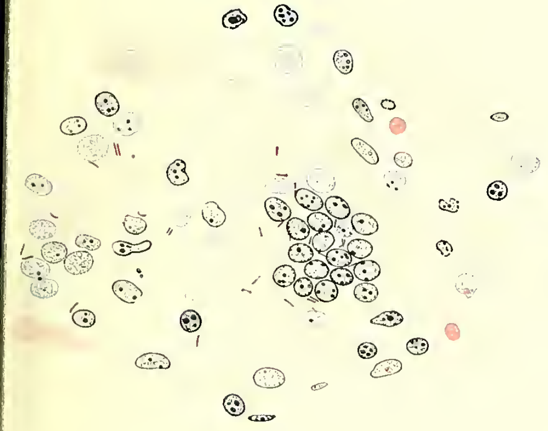

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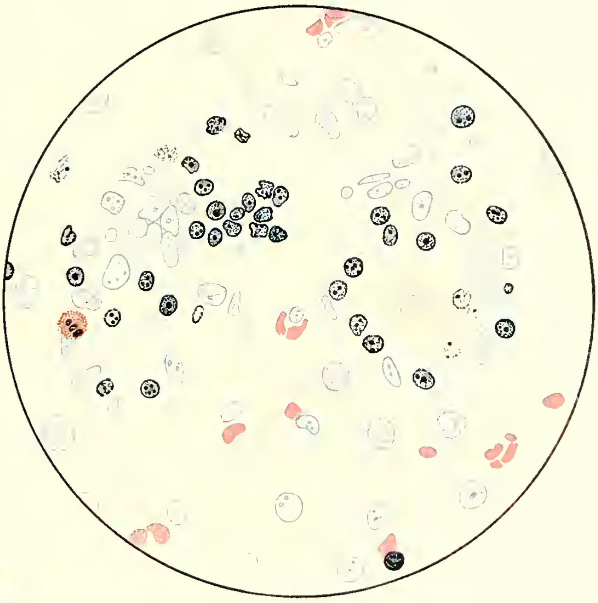

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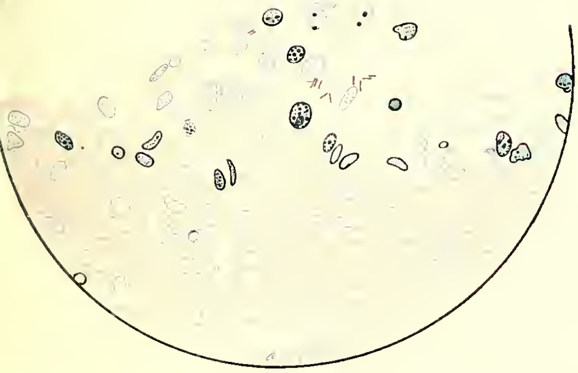

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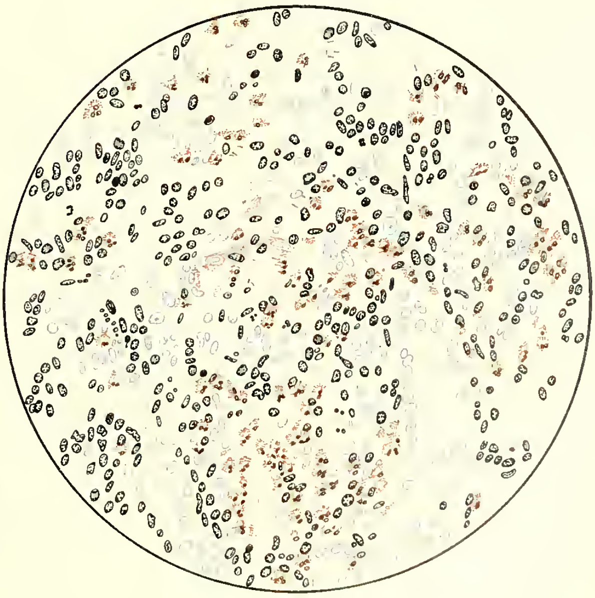

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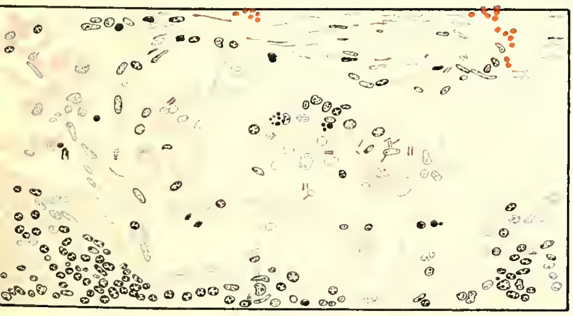

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J. R. Ford del.

Shiells & Ford, lith.

PLATE III.

STAIN.—Carbol-fuchsin followed by eosin and methylene blue.

Fig. 1. × 537. Local lesion of Calf 873, three days after subcutaneous inoculation with 50 mg. of culture of H 26. K.M. (a human virus of relatively low virulence for the bovine). The lower half of the figure shows a dense mass of multinuclear leucocytes (including mononuclear forms of the finely granular oxyphil) converging upon the inoculated culture, the greater bulk of which lies below the area illustrated. The leucocytes, many of which contain several bacilli, fail to take a granular stain, and also show signs of nuclear disintegration. The upper half of the figure is taken from the same section, at a short distance further away from the inoculated mass of culture. It shows dilation of the lymphatic channels and swelling of the endothelium and connective tissue corpuscles. The finely granular leucocytes, which are converging in the direction of the culture mass below, stain well, in contrast to the leucocytes in the lower half of the figure ; they have not ingested any of the bacilli present.

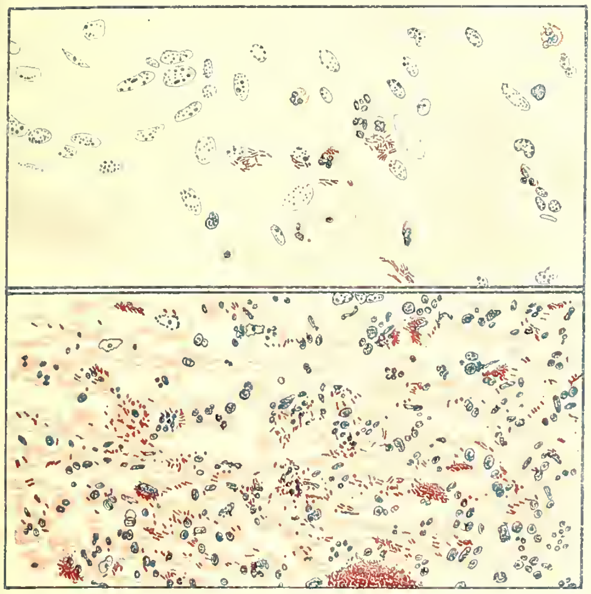
Fig. 2. × 610. Thoracic gland of Calf 288, killed 14 days after subcutaneous inoculation with 50 mg. of culture of B XIX. (a typical bovine virus). The lesion, which is typical of those produced by highly virulent bacilli, is commencing to break down, and the disintegrating tissue shows an abundant infiltration of finely granular leucocytes. It will be noted that relatively few of these cells contain bacilli.

Fig. 3. × 610. Lung of Pig 150, 14 days after subcutaneous inoculation, in the ear, with 20 mg. of culture of B IV. (a typical bovine virus). An early lesion in the lung. Under a low power such lesions appear as minute foci of partial consolidation, without any particular structure characteristic of a tubercle. Under a high power there is seen swelling of the epithelial and endothelial cells and some infiltration with wandering cells. In this case the wandering cells are mostly multinuclear leucocytes. Two of the leucocytes in the centre of the field contain bacilli. In the pig the attraction of such leucocytes to sites where tubercle bacilli occur is particularly rapid, and the leucocytes ingest the bacilli rather more frequently than in the calf or the rabbit. The essential changes in the endothelial and epithelial cells of the lung and the infiltration of wandering cells are the same as in the liver (see Plate I.) ; the difference in general appearance is due to the difference between the loose structure of the lung and the compact structure of the liver.

Fig. 4. × 530. Lung of Calf 312, killed, when in apparently good health, 90 days after subcutaneous inoculation with 50 mg. of culture of P IV (a virus of porcine origin and of lower virulence than bovine viruses). The illustration shows a portion of a caseous tubercle of progressive type. In the upper, caseous, part of the field it will be noted that :—(1) bacilli are numerous, (2) in addition to the broken-down cells, there is a fresh invasion of multinuclear leucocytes, (3) a few of these leucocytes contain bacilli. In the lower half of the figure it will be noted that the cellular boundary of the tubercle is being invaded by bacilli from the caseous area.

Fig. 5. × 215. *Fig. 6.* × 600. Kidney of a rabbit, 15 days after inoculation in the vein of an ear with 1 mg. of culture of B III. (a typical bovine virus). An early tubercle, showing its relation to the renal epithelium. Fig. 6 shows a higher magnification of the lesion seen in Fig. 5. The granular epithelium of the secretory tubules is conspicuous. In the lesion are seen degeneration of granular renal cells, some of which contain bacilli, and increase of connective tissue corpuscles. The glomerulus, at the bottom of Fig. 5, is unaffected.

PLATE III.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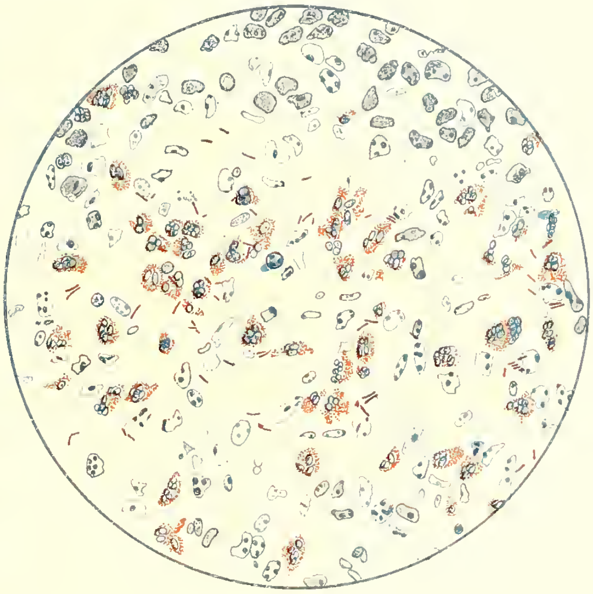
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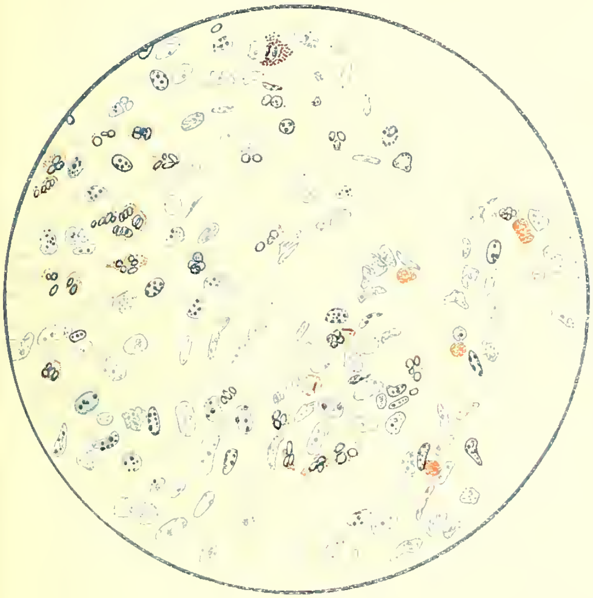
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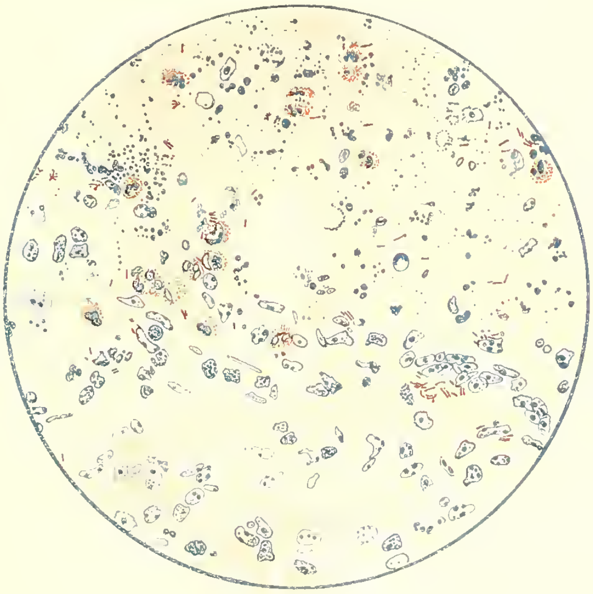
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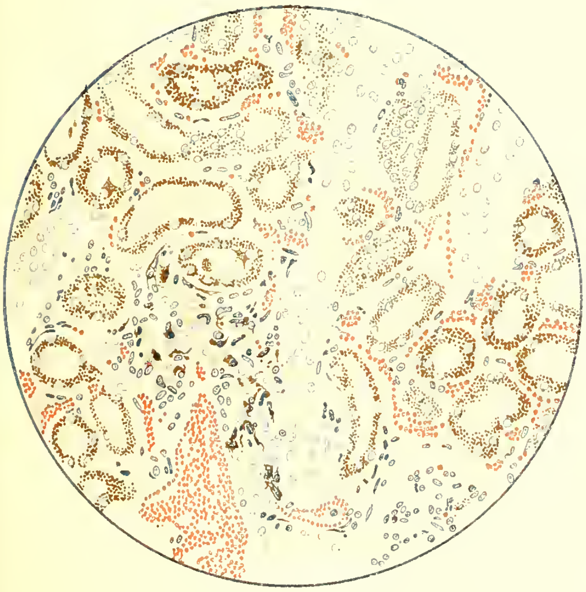
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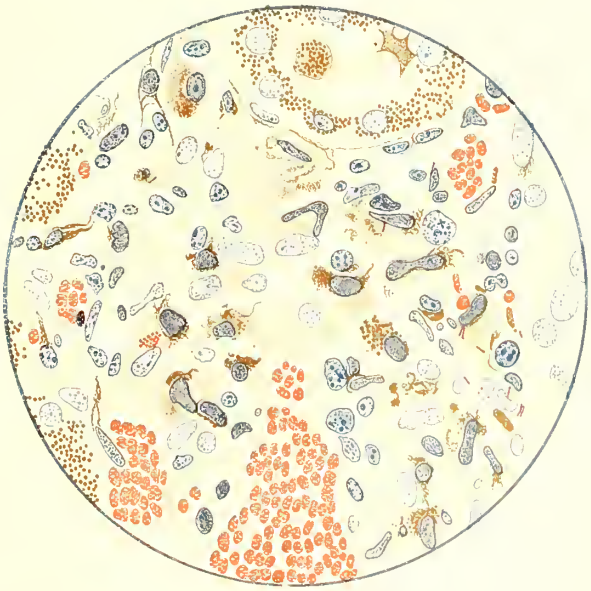
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J. R. Ford del.

Shell. & Ford. lith

PLATE IV.

STAIN.—With the exception of Fig. 1, which is stained with eosin and methylene blue alone, the specimens are stained with carbol-fuchsin, eosin, and methylene blue.

Fig. 1. × 112. Lung of Rabbit 488, killed 63 days after inoculation, partly intravenously and partly intraperitoneally, with a total of 140 mg. of killed culture of bacilli of bovine origin. This figure is a representative example of numerous lesions found in the lungs. The material which stains strongly with eosin bears a curious resemblance to actinomyces. The lesion as a whole is abundantly infiltrated with finely granular oxyphil leucocytes. As carbol-fuchsin has not been applied, no bacilli are stained.

Fig. 2. × 865. From the same lung as Fig. 1, the specimen having been stained with carbol-fuchsin before counterstaining with eosin and methylene blue. A portion of one of the actinomyces-like nodules. The club formation may perhaps be attributable to dissolved constituents of the large number of dead bacilli inoculated. Some bacilli not yet disintegrated are seen in the lower part of the field. The abundance of multinuclear leucocytes, which are shedding their granules, suggests that the disintegration of the dead bacilli is due to the digestive action of these cells.

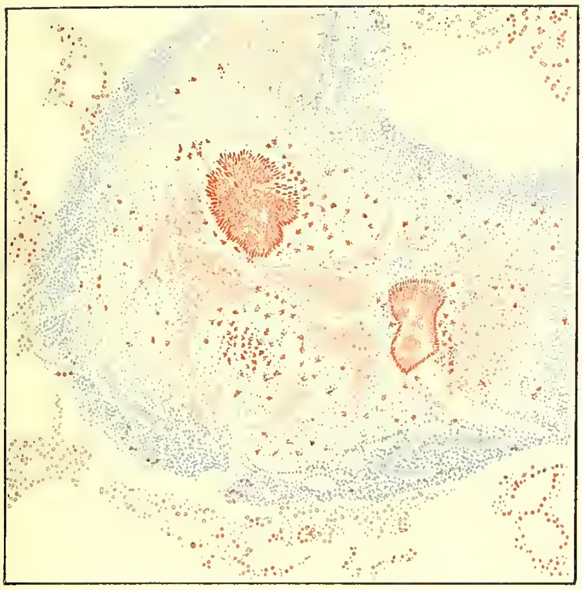
Fig. 3. × 240. Lung of Rabbit 231, killed 90 days after intraperitoneal inoculation with 1 mg. of culture of the human sputum virus H 17. Sp.B. (through Calf 265). A typical patch of tubercular consolidation with commencing caseation. Note the abundant infiltration of multinuclear leucocytes, which seem specially attracted to the breaking down tissue. The lesion is completely avascular. Some fragments of coagulated blood are seen in the lower half of the figure, to the left. The disease in the lungs has been of a relatively mild, slowly progressive type. Bacilli are scarce throughout the lung ; none are present in the field illustrated.

Fig. 4. × 294. Lung of Goat 65, which died 38 days after subcutaneous inoculation with the human lupus virus H 53. D.H. (*a*), a virus of not very high virulence. This shows another type of pulmonary tuberculosis where, with the exception of the areas occupied by "giant cells," the tissue remains highly vascular. Bacilli are scanty.

Fig. 5. × 275. Lung of Pig 80, killled 131 days after feeding with 10 million bacilli derived from B IV (a typical bovine virus). The illustration is taken from the broad, cellular margin of a chronic tubercle, the caseous portion of the tubercle being below the area illustrated. The figure shows the tendency to vascularisation of a chronic tubercle by proliferation of endothelium from pre-existing blood-channels.

Fig. 6. × 537. Lung of Calf 448, killed 104 days after subcutaneous inoculation with 50 mg. of P XIV, a porcine virus of fairly high virulence. The illustration shows a vascular type of lesion which is common in severe infections. The close proximity of bacilli to the blood-stream, especially in the lower part of the figure to the left, will be noted. Compare Fig. 4, where the scarcity of bacilli and the tendency to "giant cell" formation are histological indications of a less severe type of infection.

PLATE IV.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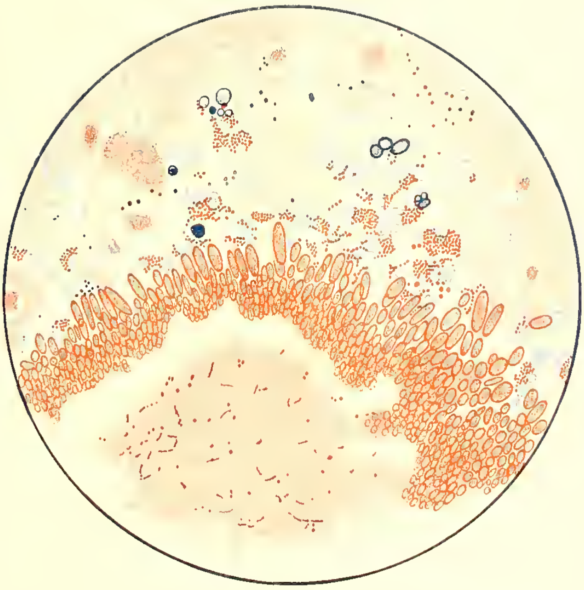
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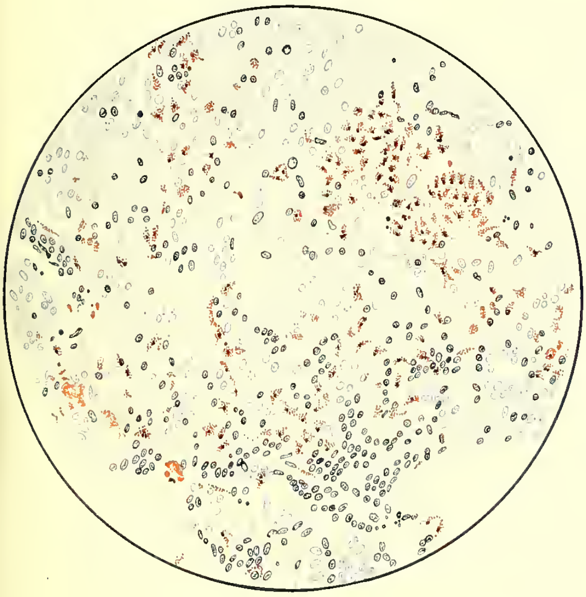
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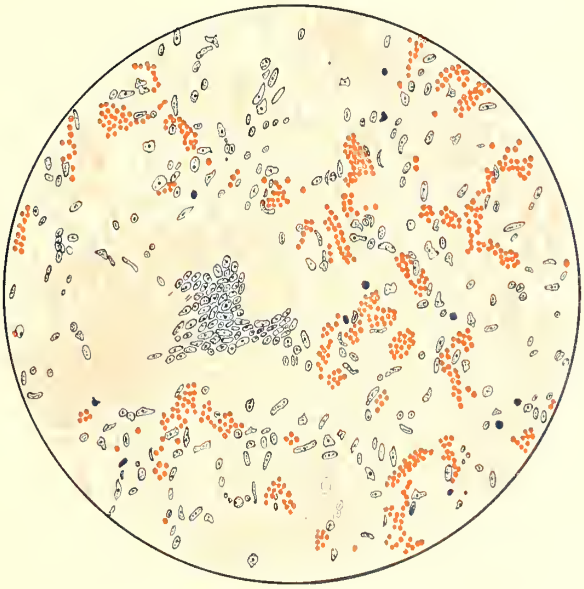
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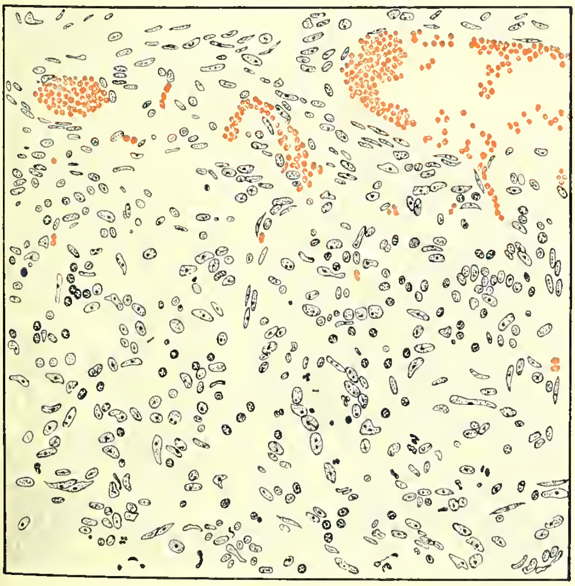
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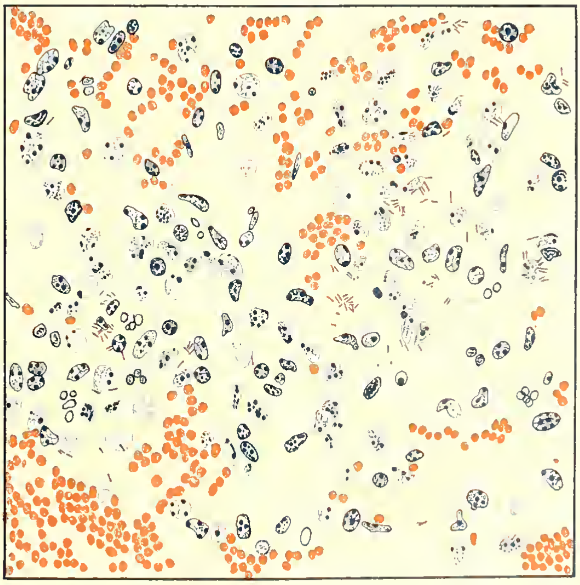
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J.R.Ford del.

Shiells & Ford, lith.

PLATE V.

STAIN.—Carbol-fuchsin and methylene blue.

Fig. 1. × 215. Tonsil of Calf 215, which died 46 days after subcutaneous inoculation of 50 mg. of culture of P XIV, a porcine virus of fairly high virulence. The illustration shows an accumulation of bacilli in a tonsillar crypt.

Fig. 2. × 537. From the same tonsil as Fig. 1. Bacilli penetrating, and others internal to, the tonsillar epithelium.

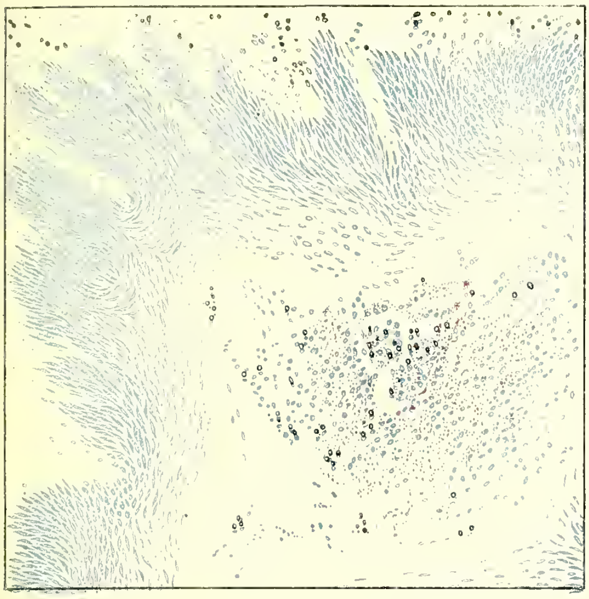
Fig. 3. × 150. From the small intestine of Monkey 73, which died 89 days after subcutaneous inoculation with 0·01 mg. of culture of H 61. E.C. (brain), a human virus of low virulence for the bovine. The specimen shows enormous numbers of bacilli in the lumen of the gut (left half of the figure) and bacilli passing between the villi (right half of figure).

Fig. 4. × 600. From the same specimen as Fig. 3. Transverse section of villi showing invasion of bacilli.

Fig. 5. × 400. Udder of Cow 74, which died 299 days after intramammary inoculation of bacilli derived from B I (a typical bovine virus). The specimen is taken from an affected portion of the mammary tissue showing early infection. Note :—(1) The high vascularity, (2) the interstitial infiltration with bacilli, (3) the entrance of bacilli into the glandular epitheleum, and (4) the excretion of bacilli into a mammary tubule. It is of importance to note that lesions such as this, which are obviously unrecognisable clinically, excrete numerous bacilli.

Fig. 6. × 215. From the same udder as Fig. 5. A portion of a circumscribed, caseous tubercle, the caseous centre being to the right. The specimen shows :—(1) The large number of bacilli which such a tubercle may contain, (2) the vascularity of the cellular margin, and (3) the close proximity of the bacilli to patent blood-vessels. This is an example of a circumscribed tubercle from which dissemination may readily occur.

PLATE V.



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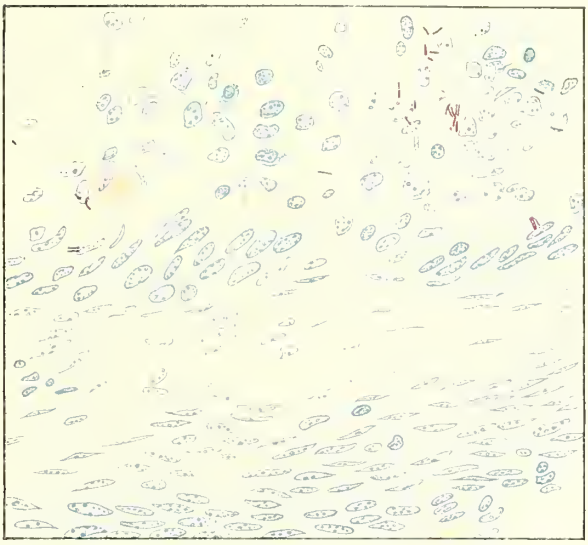

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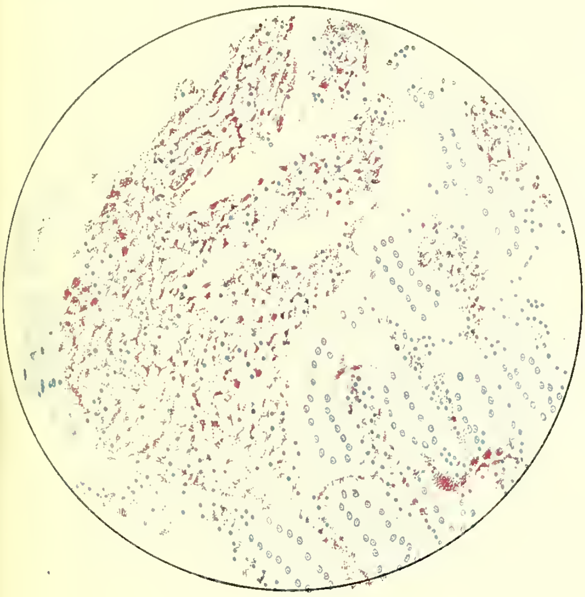

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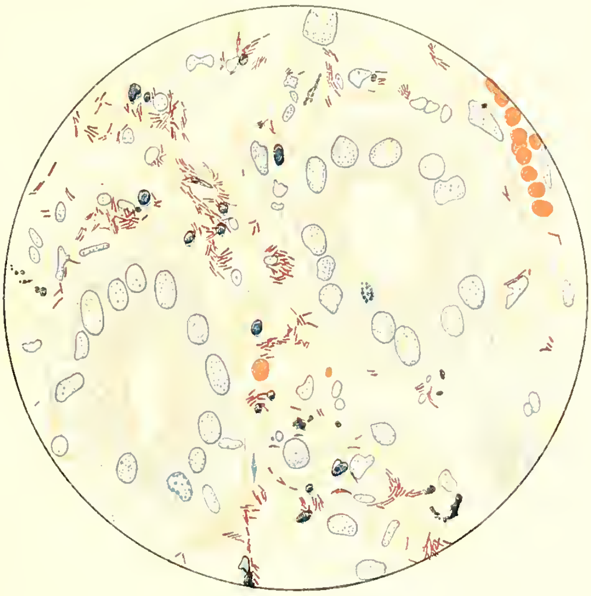

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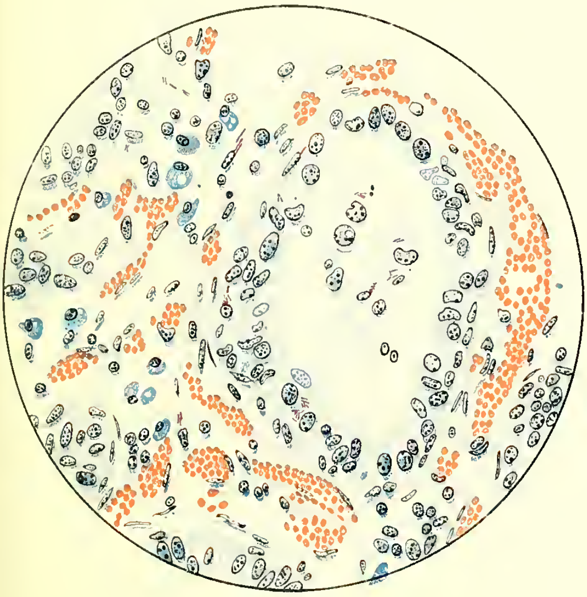

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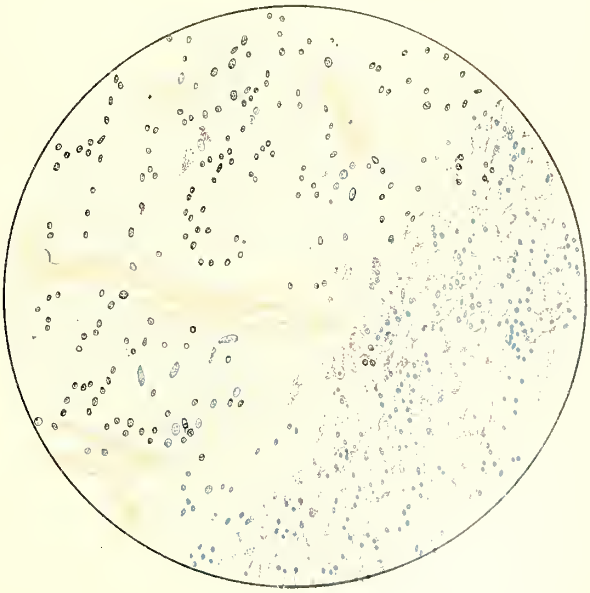

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PLATE VI.

STAIN.—Figs. 1, 3, 6, and 7 are stained with carbol-fuchsin, eosin, and methylene blue; Figs. 2, 4, and 5 are stained with carbol-fuchsin and methylene blue.

Fig. 1. × 215. Udder of Cow 3, killed 63 days fter intramammary inoculation with a culture of H 1. C.M. (a human sputum virus of relatively low virulence for experimental animals). The illustration shows portions of two typical tubercles of chronic type. At the bottom of the figure a glandular tubule has survived. Bacilli, which were present in the caseous centres of many of the lesions, do not occur in this figure.

Fig. 2. × 400. Udder of Cow 4, killed 34 days after intramammary inoculation with B II (a typical bovine virus). Wall of milk sinus, the lumen of the sinus being in the lower half of the figure. Note the vascularity and abundance of bacilli. From breaking down tissue such as this bacilli may readily escape into the milk stream in large numbers.

Fig. 3. × 215. Lung of Calf 365, killed 61 days after subcutaneous inoculation with 100 million bacilli of H 22. J.P. (a human virus of relatively low virulence for the bovine). The infection is relatively mild in type, but there is no limitation of the lesions by definite boundary zones. The greater part of the tissue is still vascularised. There is an abundant infiltration of leucocytes and lymphocytes. Compare the rather more severe lesion shown in Fig. 4, Plate IV, where multinuclear leucocytes do not occur.

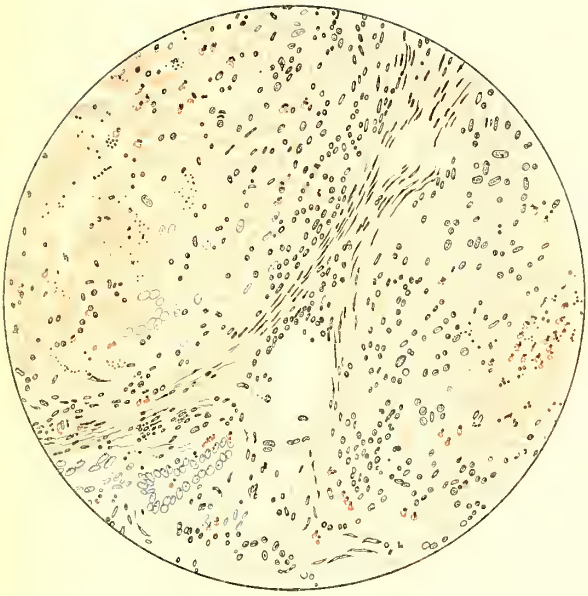
Fig. 4. × 82. Lung of Calf 525, killed 90 days after subcutaneous inoculation with 50 mg. of culture of H 7. C.M. (a human virus of high virulence for young calves—Calf 525 was older than the average). An obviously retrogressive tubercle. It is almost completely filled up with lymphocytes; there is no fibrous zone.

Fig. 5. × 82. Lung of Calf 627, killed 90 days after subcutaneous inoculation with 50 mg. of culture of H 37. O.J. (a human virus of low virulence for the bovine). An obviously retrogressive tubercle, surrounded by a densely fibroid zone.

Fig. 6. × 400. Liver of Pig 94, killed 61 days after subcutaneous inoculation with 1 mg. of culture of B IV (a typical bovine virus). The wall of a vein, with a pedunculated tubercle projecting into the lumen, illustrating the development of a tubercle from endothelium. Some of the finely granular oxyphil leucocytes are of the mononuclear type.

Fig. 7. × 235. Epididymis of Rabbit 255, killed 90 days after intraperitoneal inoculation with 50 mg. of H 40. J.G. (a human virus of relatively low virulence). A portion of the wall of a tubule is shown, with a caseous mass, crowded with bacilli, projecting into the lumen.

PLATE VI.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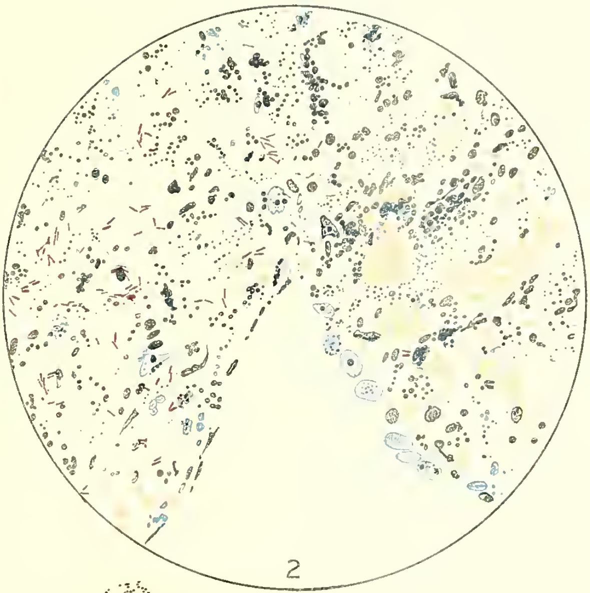
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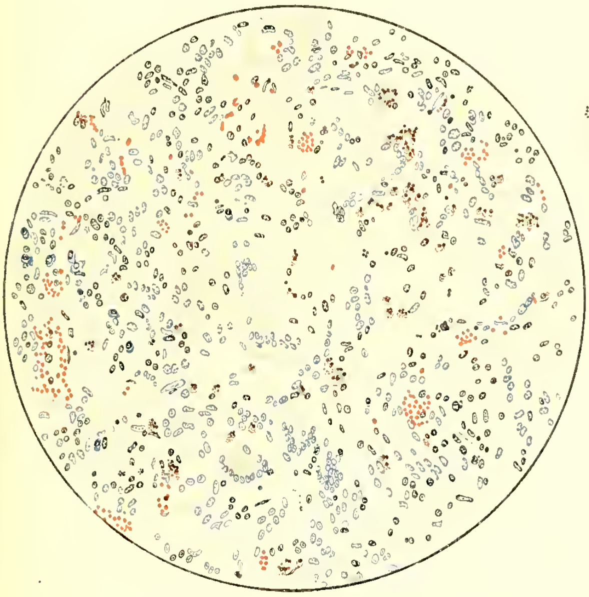
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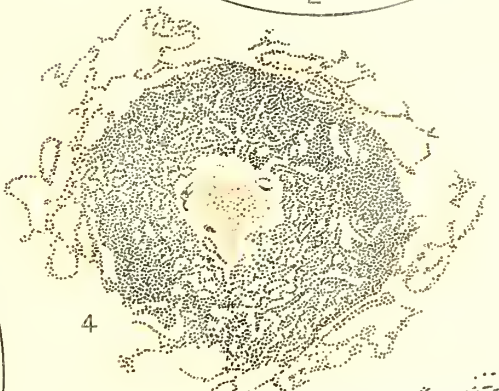
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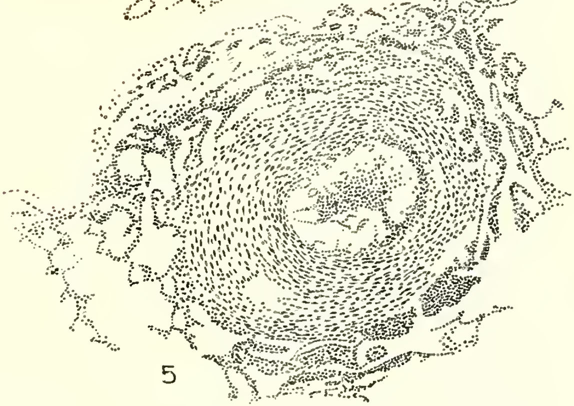
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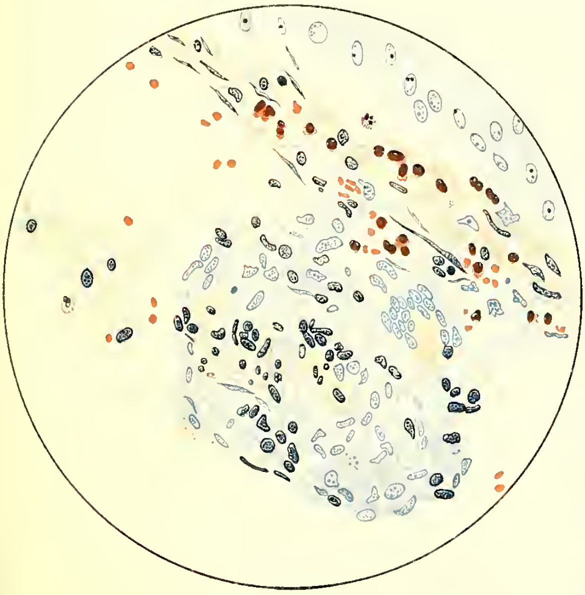
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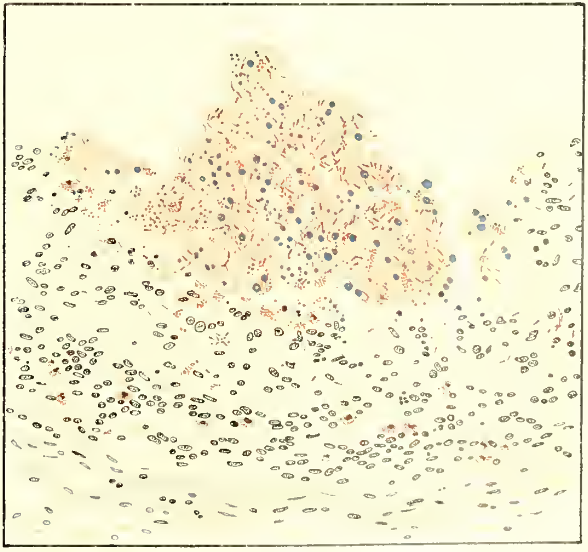
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PLATE VII.

STAIN.—For Figs. 1 and 2 carbol-fuchsin and methylene blue; for Fig. 3 Kochel's fibrin stain; for Fig. 4 Kochel's fibrin stain followed by thionin; for Figs. 5 and 6 Kochel's fibrin stain preceded by carbol-fuchsin and followed by thionin. In the reproductions the fibrin is not black enough.

Fig. 1. × 275. Lung of Chimpanzee 10, which died 113 days after feeding with 0·1 mg. of culture of B IV (a typical bovine virus). Typical caseous pneumonia, produced by feeding with a small dose of bovine bacilli.

Fig. 2. × 215. Mesenteric gland of Calf 406, killed 91 days after subcutaneous inoculation with 10 mg. of culture of E II (an equine virus of intermediate virulence). The illustration shows a portion of a caseous tubercle with a broad fibrous margin. The features of special interest are the unusual abundance of bacilli within a tubercle of this type and the tendency of the bacilli to form rosettes, like avian tubercle bacilli, although the virus in question is undoubtedly of mammalian type.

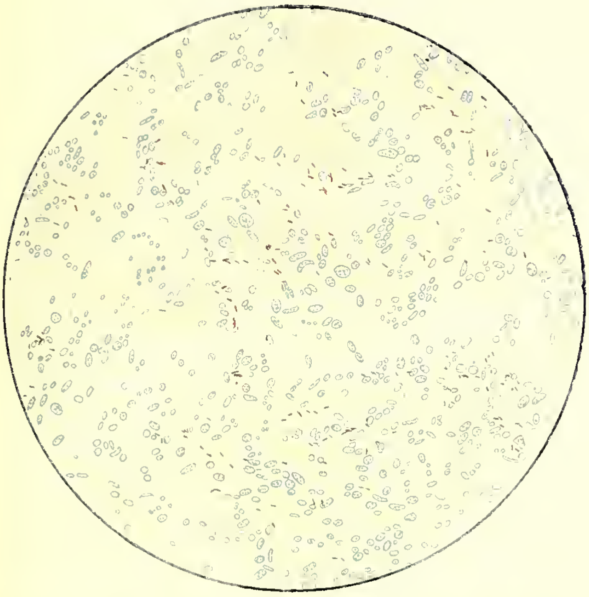
Fig. 3. × 50. Iliac gland of Cow 500, killed when moribund 32 days after intramammary inoculation with B II (a typical bovine virus). Early deposits of fibrin, which are stained black. This is typical of acute infection, and shows how the early lesions may be readily picked out under a low power. When the specimens are also stained for bacilli, it is found, in early stages of acute infections such as this, that bacilli occur in association with the deposits of fibrin.

Fig. 4. × 50. Udder of Cow 75, killed when very ill 34 days after intramammary inoculation with bacilli from H 14. F.S. (a human virus of high virulence for the bovine). Early deposits of fibrin in acute infection of the udder.

Fig. 5. × 400. Liver of Calf 711, which died 33 days after subcutaneous inoculation with 50 mg. of culture of H 16. J.H. through Calf 317 (a human virus which in this experiment proved to be of high virulence). The figure shows the association of bacilli with deposits of fibrin in acute infection of the liver.

Fig. 6. × 400. Liver of Calf 544, which died 33 days after intravenous inoculation with 10 mg. of culture of A VIII (a typical avian virus). Acute infection, characterised by abundant deposit of fibrin, produced in the liver of the calf by intravenous inoculation with avian bacilli.

PLATE VII.



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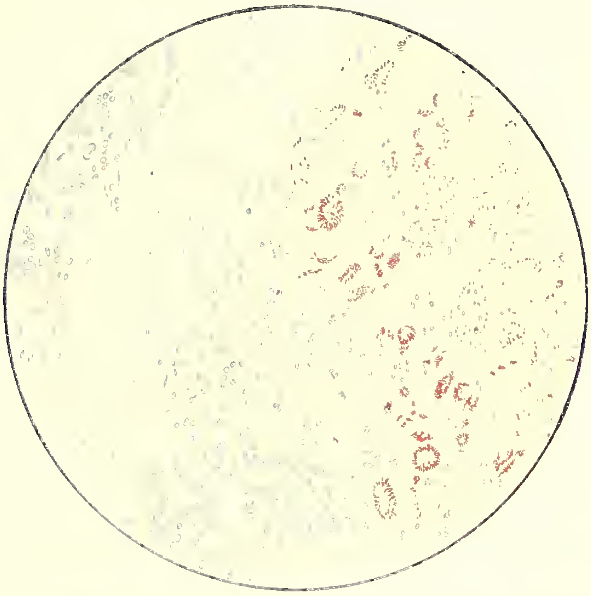

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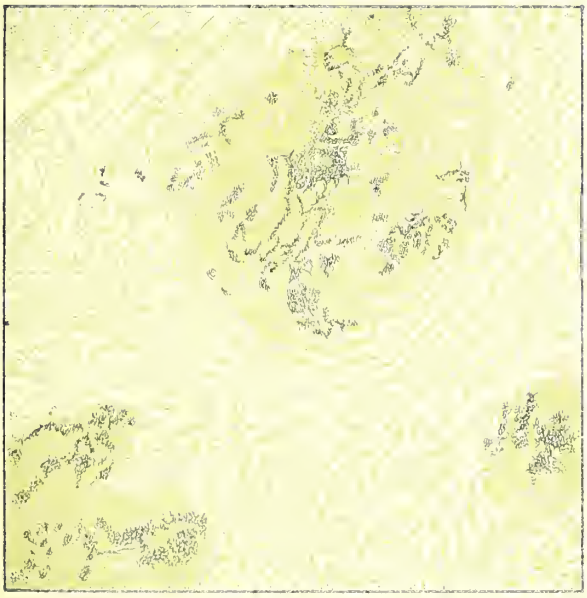

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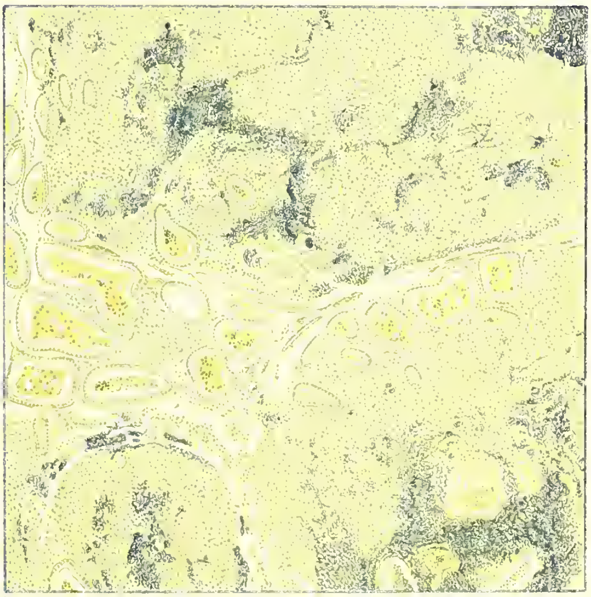

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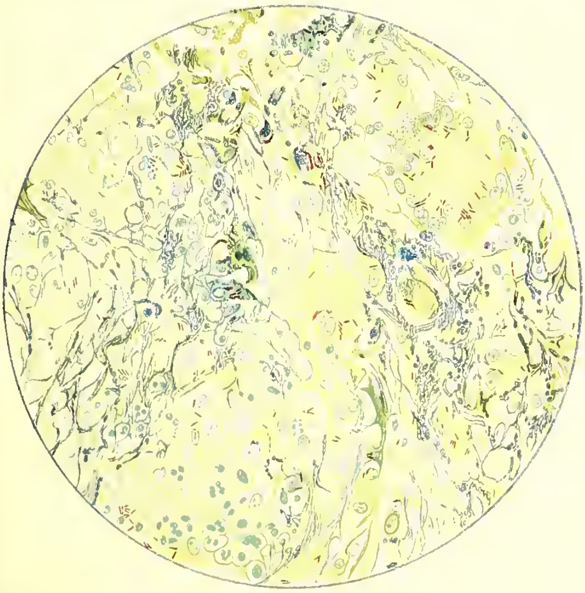

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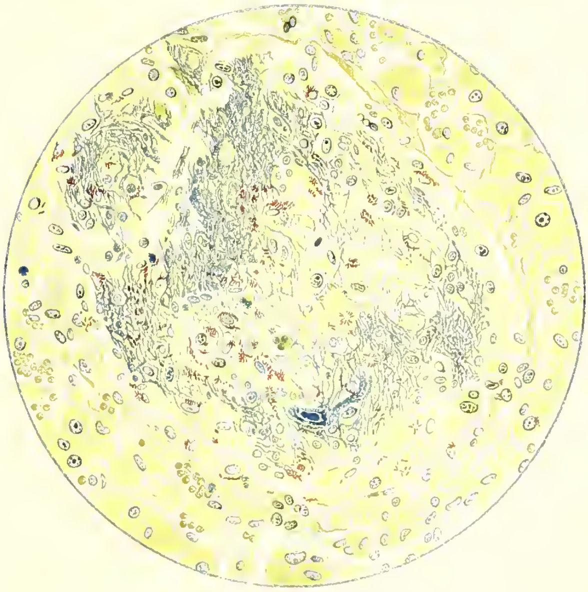

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PLATE VIII.

STAIN.—Kochel's stain for fibrin, which, in Figs. 4–6, is preceded by carbol-fuchsin and followed by thionin. In the reproductions the fibrin is not black enough.

Fig. 1. × 50. Lung of Calf 711. (The liver is shown in Fig. 5 of Plate VII.) Low power view of deposits of fibrin in acute infection of the lung.

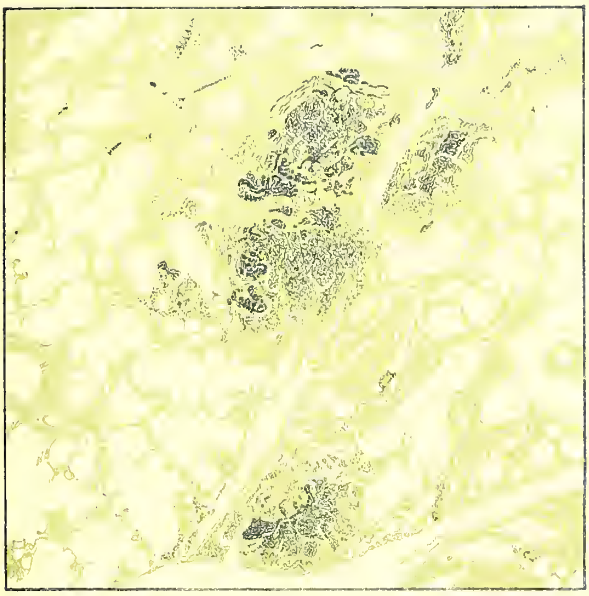
Fig. 2. × 150. Lung of Calf 551 (a bull-calf 10 months old), which died 21 days after intravenous inoculation with 100 mg. of culture of H 25. A.T. (a human virus of low virulence for the bovine when inoculated subcutaneously). The lungs were acutely infected. The specimen shows the thickened pleura with fibrinous deposit amongst unstained exudate. The margin of the alveolar tissue is seen at the bottom of the figure.

Fig. 3. × 112. Lung of Chimpanzee 18, which died 116 days after feeding with ·01 mg. of culture of B IX. (a typical bovine virus). Acute infection of the lung, with abundant deposit of fibrin, after feeding with a small dose of bovine bacilli.

Fig. 4. × 600. Lung of Chimpanzee 3, which died 77 days after feeding with 1 mg. of culture of H 25. A.T. (a human virus of low virulence for the bovine). A high power view showing acute infection with abundant deposits of fibrin, after feeding with bacilli of human origin.

Fig 5. × 610. Lung of Calf 93, which died 73 days after intraperitoneal inoculation with 600,000 bacilli from H. 2. Sp. A. through Calf 79 (a human sputum virus of high virulence for the bovine). A high power view showing deposits of fibrin in the bovine lung owing to acute infection produced by a small (intraperitoneal) dose of virulent bacilli derived from human sputum.

Fig. 6. × 400. Lung of Calf 544. (The liver is shown in Fig. 6 of Plate VII.) Fibrin formation produced in the calf's lung by acute infection due to intravenous inoculation of 10 mg. of culture of avian bacilli. In addition to the fibrin, the catarrhal exudate filling the alveoli is well shown.



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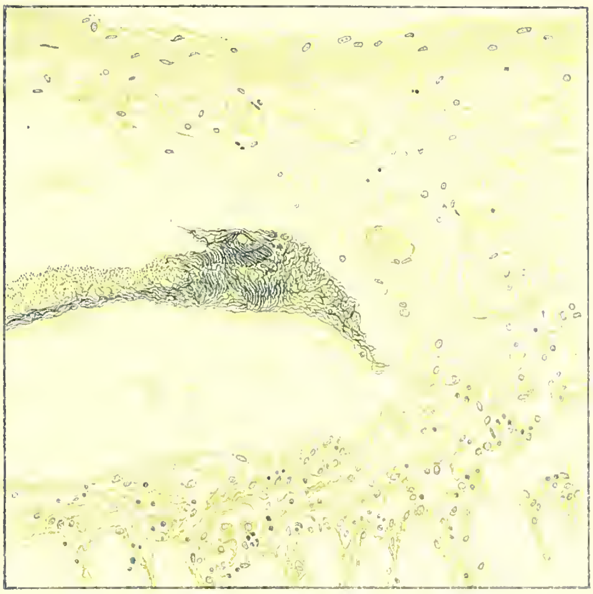

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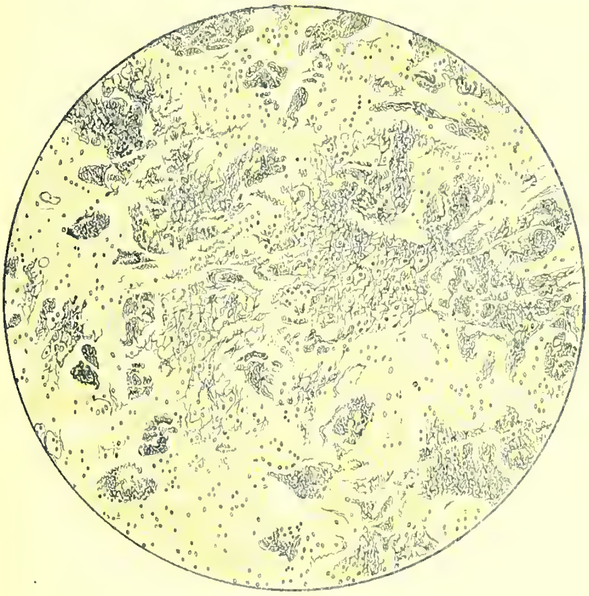

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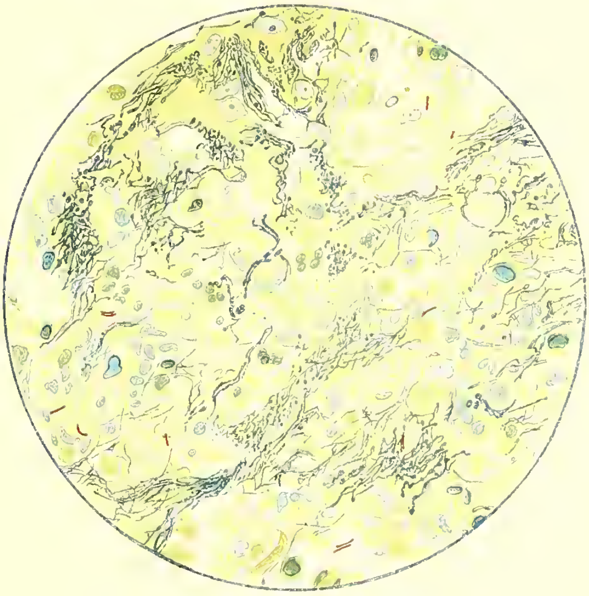

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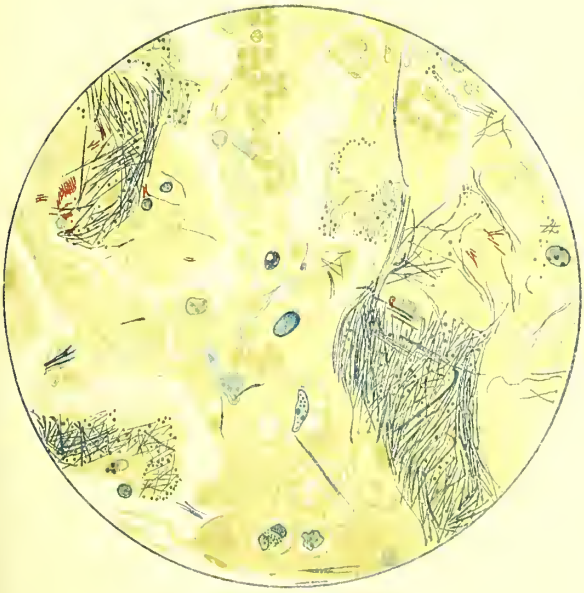

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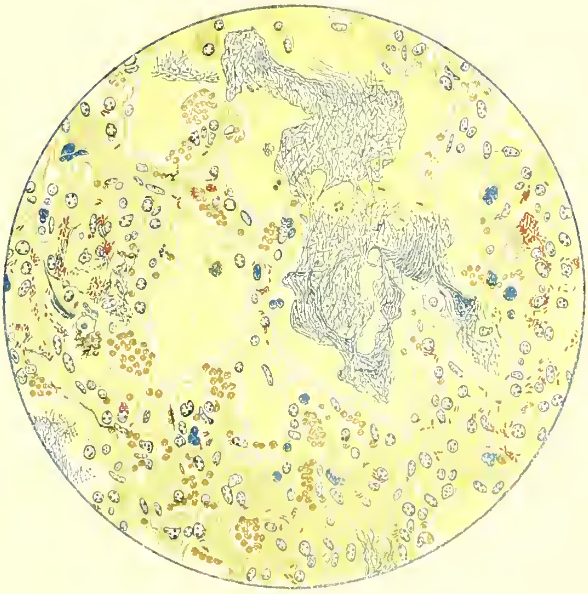

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PLATE IX.

STAIN.—Pappenheim's stain for plasma cells. Figs. 5 and 6 have been previously stained with carbol-fuchsin.

Fig. 1. × 600. Bronchial gland of Calf 101, killed when moribund 41 days after intravenous inoculation with 48,000 bacilli from H. 7. C.M. (a human virus of high virulence for the bovine). This figure is taken from a normal (medullary) portion of the lymphatic gland, and shows the characteristic appearances of large lymphocytes when stained by Pappenheim's method.

Fig. 2. × 600. From a lesion in the cortical portion of the same gland as Fig. 1. The illustration shows that cells which react to Pappenheim's stain do not take an active part in the formation of tubercles but disappear from the lesions, which are developed from the glandular frame-work. Outside the lesion, to the left, normal large lymphocytes are seen.

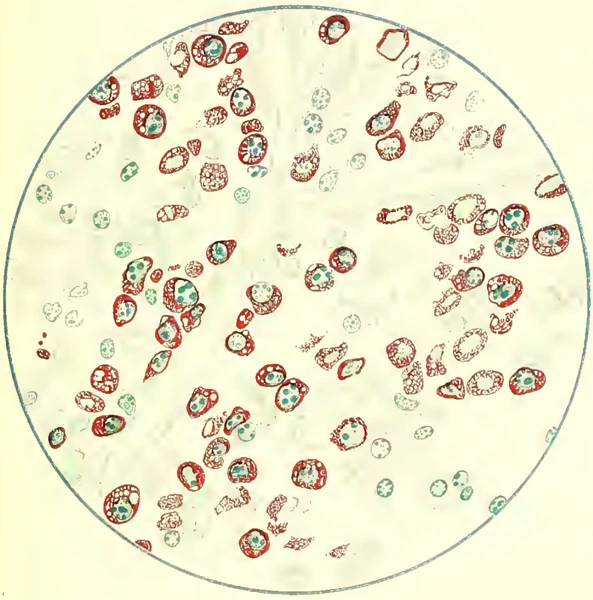
Fig. 3. × 275. Liver of Pig 150, killed 14 days after subcutaneous inoculation with 20 mg. of culture of B IV. (a typical bovine virus). The illustration shows the infiltration of plasma cells into an early tubercle. It will be noted that :—(1) The lesion shows very little breaking down (*i.e.*, irritant) material ; (2) the plasma cells appear to be passing in from the interlobular tissue ; (3) they vary in outline from spherical to spindle-shaped.

Fig. 4. × 600. Liver of Rabbit 1283, killed 29 days after intravenous inoculation with 50 mg. of killed culture of bovine bacilli. Peripheral infiltration of plasma cells surrounding the relatively mild type of lesion which is produced by dead bacilli. When in proximity to lesions where more active tissue destruction is at work, plasma cells rapidly become disintegrated.

Fig. 5. × 600. Local lesion of Calf 292, killed eight days after subcutaneous inoculation with 50 mg. of culture of B IV. (a typical bovine virus). The figure illustrates the disintegration of plasma cells in the neighbourhood of tubercle bacilli and their inability to ingest such bacilli.

Fig. 6. × 600. Local lesion of Calf 510, killed 96 days after subcutaneous inoculation with 50 mg. of culture of Z III. (a virus of relatively low virulence derived from an antelope). The lesion is of retrogressive type and is undergoing fibroid change, although bacilli are still present, chiefly within connective tissue cells. The infiltrating plasma cells are normal in appearance, in contrast to the plasma cells found in the acute infection shown in Fig. 5.

PLATE IX.



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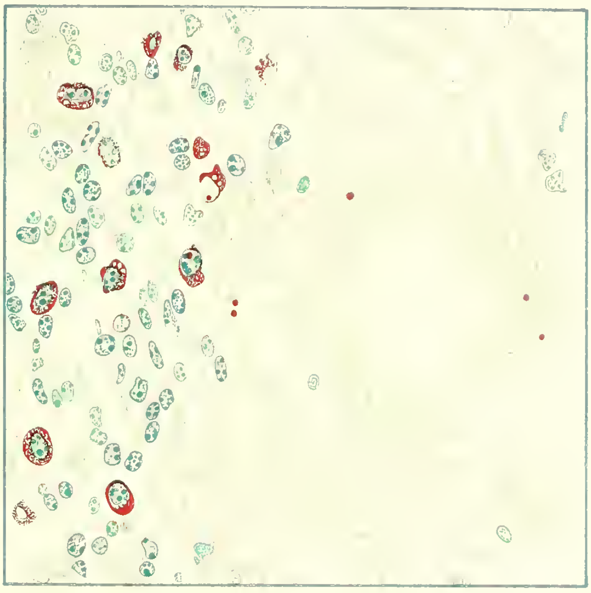

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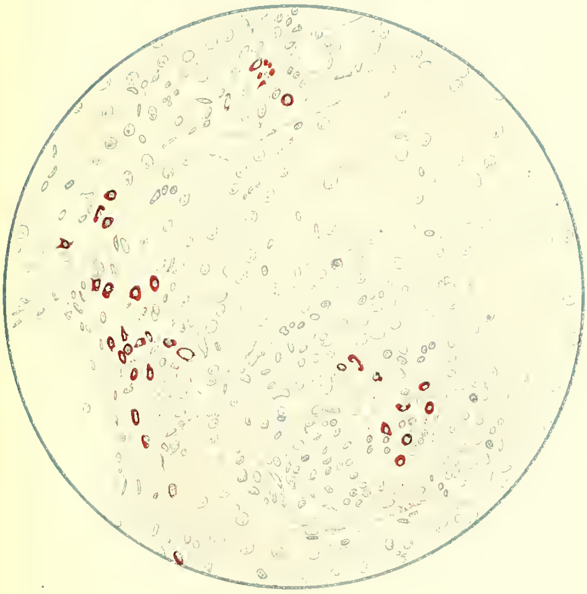

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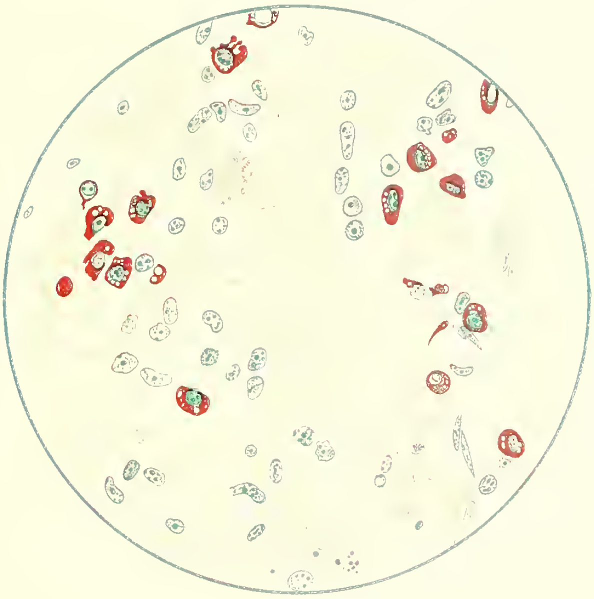

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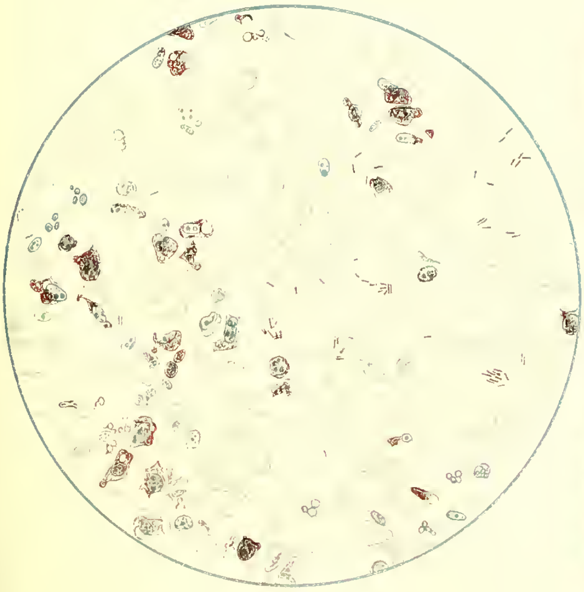

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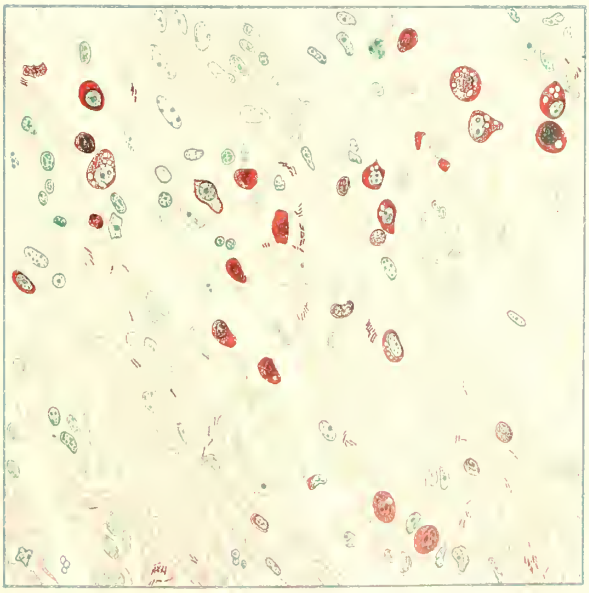

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J.R.Ford

PLATE X.

STAIN.—Pappenheim's stain for plasma cells.

Fig. 1. × 400. From the same local lesion as Fig. 6, Plate IX, but nearer to the periphery. The outermost zone (upper part of figure) consists of a broad and dense layer of fibrous tissue. Internal to this (lower part of figure) is a zone of less compact connective tissue, abundantly infiltrated with plasma cells. No bacilli are present in this region. Passing further inwards, below the lowest part of the figure, would be found the area shown in Fig. 6, Plate IX.

Fig. 2. × 240. Lung of Goat 28, which died 91 days after subcutaneous inoculation with ·01 mg. of culture of B V (a typical bovine virus). The specimen shows the infiltration of plasma cells in the wall of a typical caseous, circumscribed tubercle of the lung. The caseous interior of the tubercle is upwards and to the left.

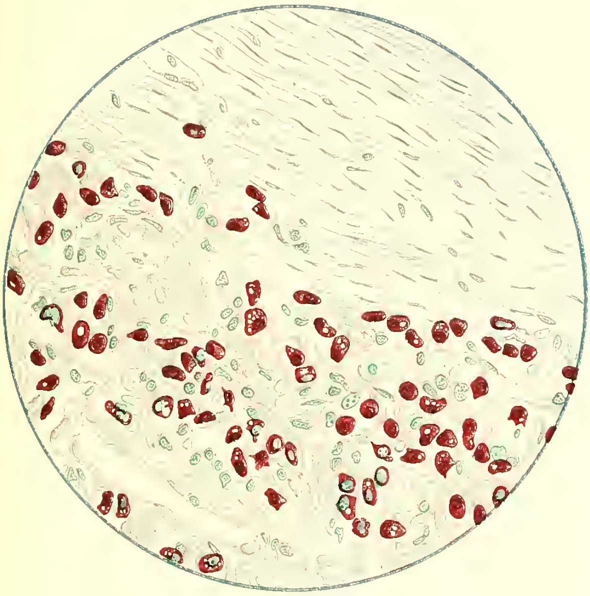
Fig. 3. × 340. Kidney of Calf 301, killed when very ill 33 days after subcutaneous inoculation with 540 million bacilli from H 13. A.D. (a human virus which in this experiment was of high virulence for the bovine). Early tuberculosis of the kidney with the characteristic interstitial infiltration and commencing disintegration of the adjacent renal epithelium. The specimen shows the participation of plasma cells in the infiltration.

Fig. 4. × 240. Lung of Calf 312, killed in apparently good health 90 days after subcutaneous inoculation with 50 mg. of culture of P IV (a porcine virus of relatively low virulence for cattle). Plasma cells in the areolar tissue surrounding a small bronchus. Plasma cells originate in such situations as this (and also in the connective tissue area surrounding blood-vessels) and then migrate towards the lesions caused by the tubercle bacillus. The plasma cells first appear as elongated cells which are distinguished from the fixed connective tissue cells by their reaction to the pyronin stain; when in process of migration, their protoplasm swells up and tends to assume a more spherical form.

Fig. 5. × 340. Udder of Cow 3, killed 63 days after intramammary inoculation with culture of H 1. C.M. (a human sputum virus of low virulence for cattle). The infection of the udder was of relatively mild type, but progressive. The specimen shows the participation of plasma cells in the infiltration which is prior to the formation of definite tubercles.

Fig. 6. × 600. From the same udder as Fig. 5. A portion of the wall of a chronic tubercle of the udder, the caseous area being above the figure Transitional stages showing the transformation of plasma cells into fibroblasts.

PLATE X.



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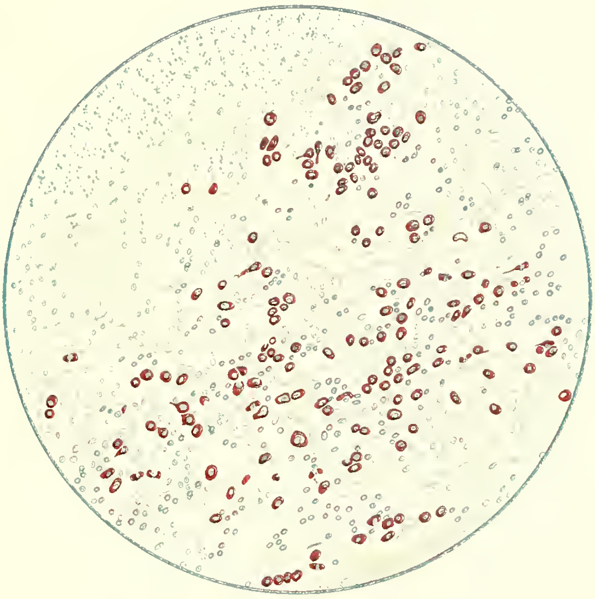

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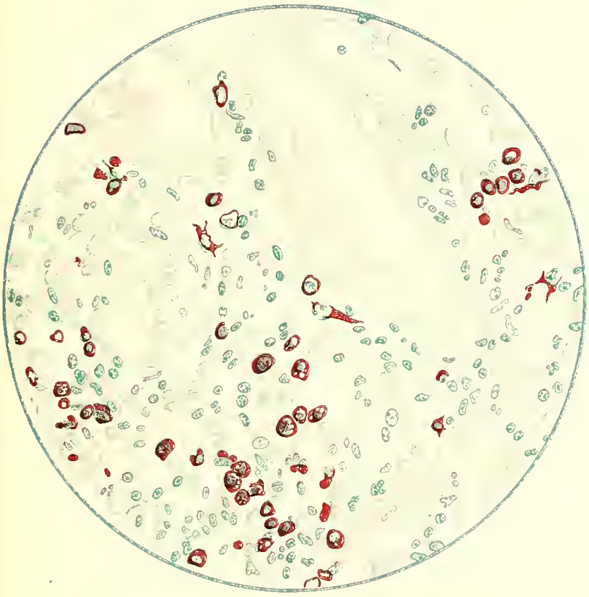

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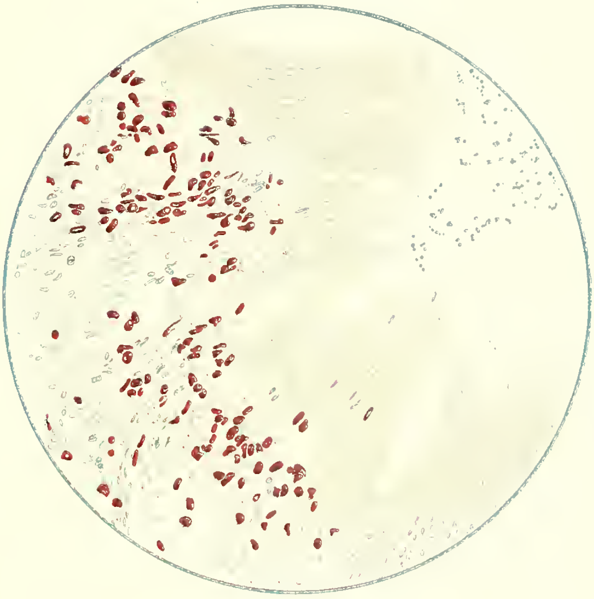

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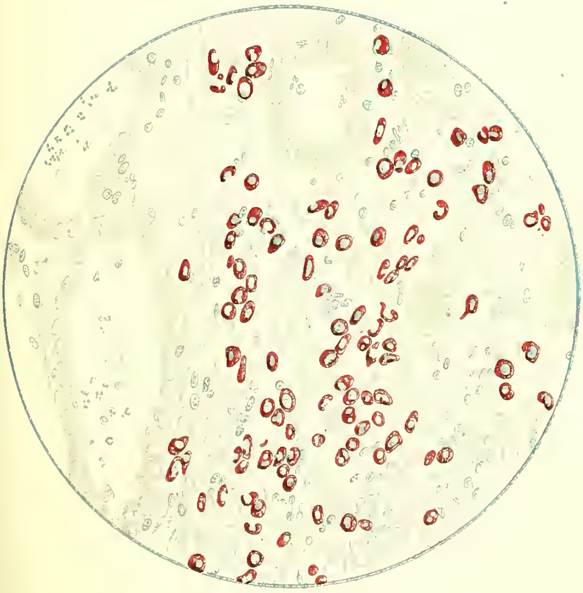

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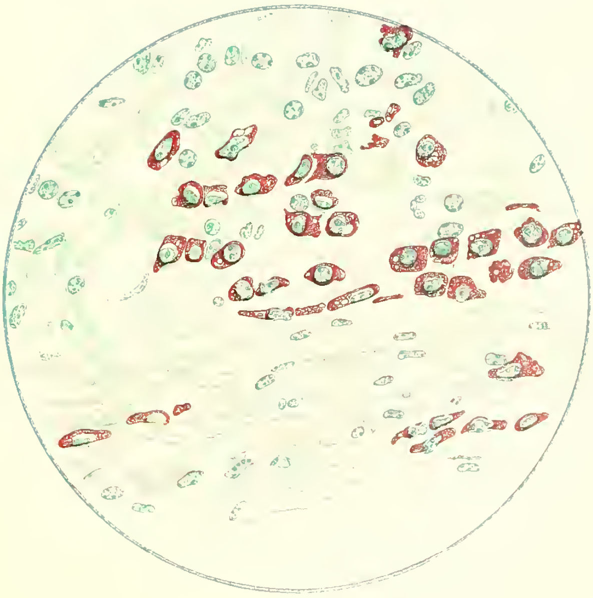

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PLATE XI.

Stain.—Carbol-fuchsin followed by eosin and methylene blue.

Magnification.—× 600, except Fig. 13, the magnification of which is ×294.

In Figs. 7, 8, 9, and 10 the blurred appearance, caused by the escape of nuclear material into the surrounding protoplasm, has not been reproduced very successfully.

Figs. 1–11 represent early lesions produced in the liver of the rabbit after inoculation in the vein of an ear with 1 mg. of culture of avian tubercle bacilli. Comparison may be made with Plate I, which shows the effects of similar doses of mammalian bacilli. In Plate XI, Fig. 9 shows a typical well-developed lesion (compare Fig. 9 of Plate I), and the preceding figures show the way in which it is produced (compare the preceding figures of Plate I).

Fig. 1. 24 hours after inoculation. A bacillus in contact with an endothelial cell, the nucleus of which is swollen and stains more darkly than is normal.

Fig. 2. 24 hours after inoculation. Two bacilli in contact with a swollen endothelial nucleus. The figure is complicated by the presence in the capillary of some circulating cells. The disintegrated cells with blue protoplasm are possibly desquamated endothelium. A multinuclear leucocyte is in contact with a bacillus.

Fig. 3. Five days after inoculation. A more advanced stage than that shown in Fig. 1. The bacilli have multiplied within an endothelial cell, the nucleus of which has divided into two.

Fig. 4. Three days after inoculation. Further splitting of endothelial nuclei ; tendency of the bacilli to grow into a rosette form.

Fig. 5. Five days after inoculation. Bacilli are present inside the endothelium ; they have also passed into the liver cells, which are beginning to be infiltrated with leucocytes and lymphocytes.

Fig. 6. Five days after inoculation. A typical rosette formation of bacilli within a liver cell. With the exception of slight swelling of the nucleus of the adjacent endothelial cell, there is no evidence of tissue reaction.

Fig. 7. Eight days after inoculation. An early tubercle, showing increase of nuclei by direct division. It is no longer possible to distinguish between endothelial and liver cell nuclei. The nuclei in the affected area stain more darkly than those of normal cells and, as shown by the diffuse blue stain, there is some escape of nuclear material into the surrounding protoplasm (compare Figs. 7–9 of Plate I). In order to bring into prominence this diffusion of the nuclear stain, which is characteristic of early lesions, more eosin has been left in the specimen, thus contrasting the affected area with the pink protoplasm and pale nuclei of the surrounding normal tissue.

Fig. 8. 11 days after inoculation. Early tubercle formation, in a more advanced stage than is shown in Fig. 7. Multiplication by direct division of liver cell nuclei and endothelial nuclei ; one liver cell nucleus with a mitotic figure. Increase of bacilli, which are notably small.

Fig. 9. From another area of the same section as Fig. 8, showing a more advanced stage of tubercle formation than the latter. Note the sharp demarcation between the affected area and the surrounding normal tissue, with no intermediate zone of inflammatory change such as is commonly found in lesions caused by mammalian bacilli. A few small lymphocytes have invaded the tubercle but only one multinuclear leucocyte. Compared with Fig. 8, there is an increase of bacilli and more breaking down of the cell protoplasm. The blurred appearance in the upper part of the lesion is due to the diffuse staining of dissolved nuclear material.

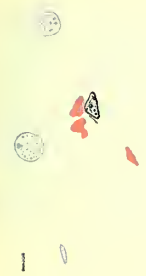
Fig. 10. From another area of the same section as Figs. 8 and 9. The changes in the fixed tissue cells are much the same as in Fig. 9, but there is much more evidence of irritant action, as shown by the invasion of multinuclear leucocytes and the presence of nuclear remains of these cells. With the exception of two in the upper part of the field (one to the left and one to the right), the leucocytes do not contain bacilli.

Fig. 11. 14 days after inoculation. An example of a "giant cell" produced by the avian bacillus. The bacilli have been growing abundantly within the "cell" and are very small. This figure may be compared with Fig. 3 of Plate II, which shows a "giant cell" produced by a mammalian bacillus.

Fig. 12. Liver of Pig. 248, which died 47 days after intravenous inoculation with 50 mg. of culture of avian bacilli (A VIII). This area is typical of the condition found in the liver of this animal. Note :—(1) the profuse growth of bacilli, with a tendency to rosette formation ; (2) the huge "giant cell" showing multiplication of nuclei by irregular longitudinal splitting ; (3) the absence of wandering cells, with the exception of a few small lymphocytes ; (4) the presence of numerous bacilli in the blood-stream.

Fig. 13. × 294. Mesenteric gland of Pig. 180, killed 105 days after subcutaneous inoculation with 50 mg. of culture of avian bacilli (A VII). A portion of a lymph space. From the endothelium which covers it a tubercle has developed and projects into the lumen. Numerous bacilli are present in the tubercle and some are also found attached to loose broken-down cells in the lymph channel. It is exceptional to find such abundant dissemination of avian bacilli in the pig after subcutaneous inoculation.

PLATE XI.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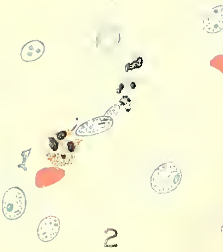
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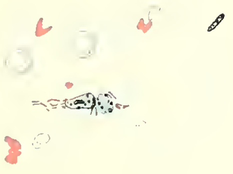
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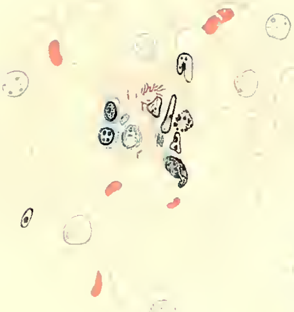
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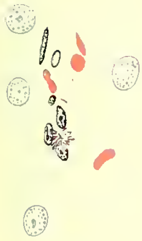
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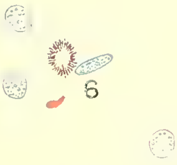
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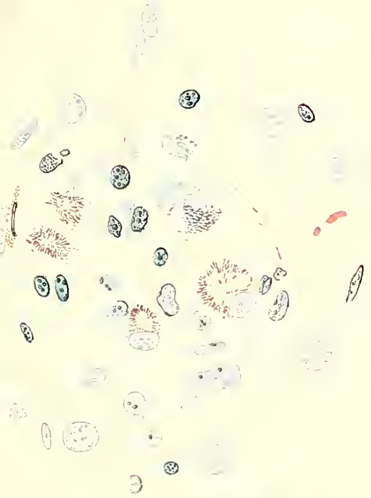
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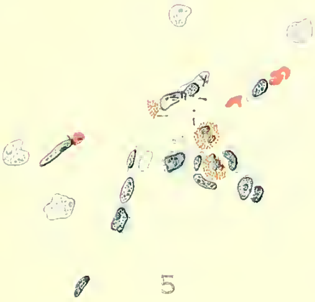
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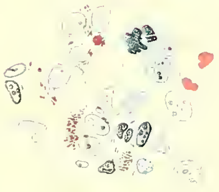
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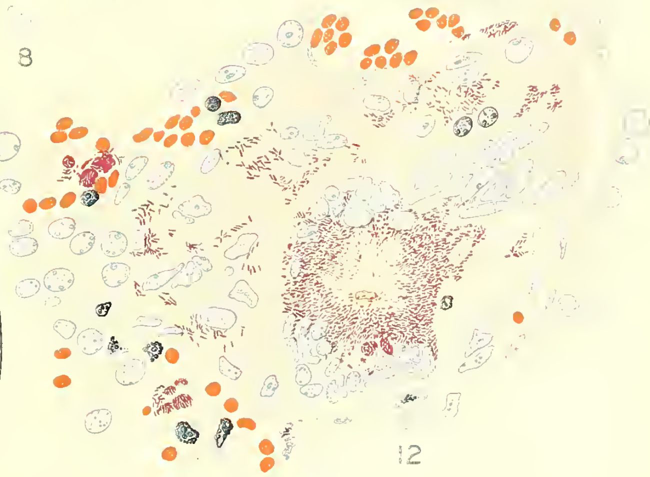
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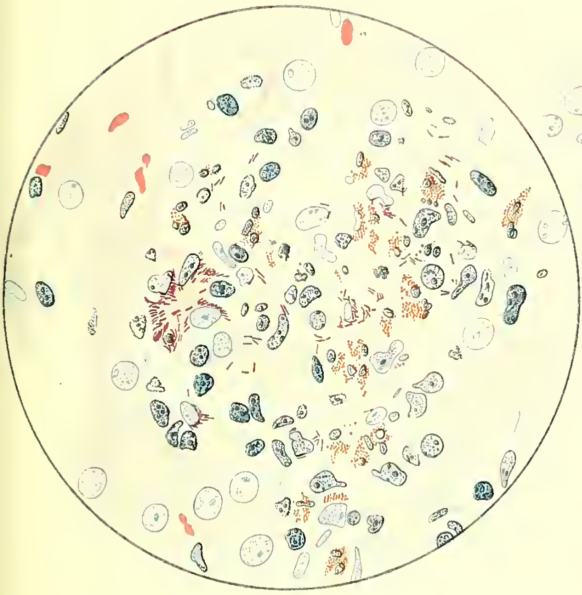
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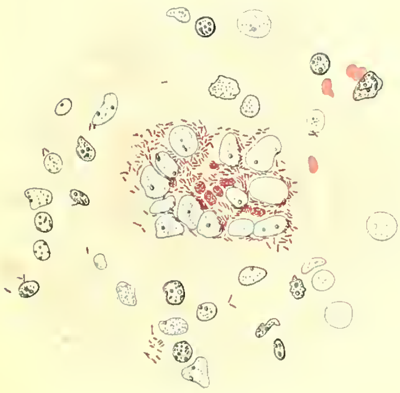
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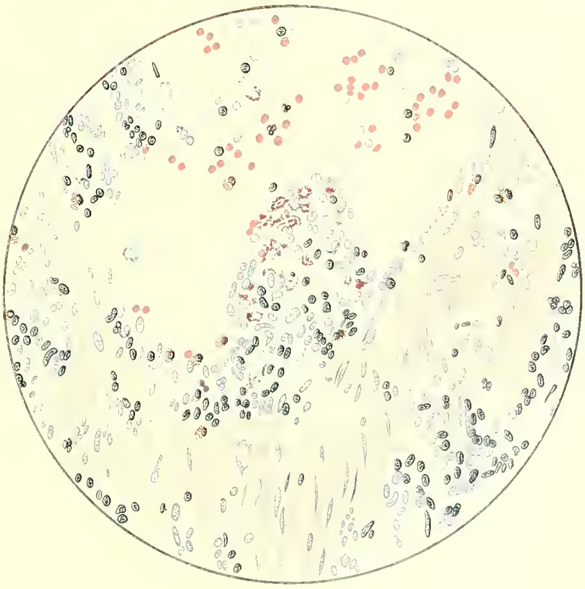
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J. R. Ford del.

Shiells & Ford, lith.

PLATE XII.

STAIN.—Figs. 1–4 are stained with carbol-fuchsin and counterstained with orange G and methylene blue; Figs. 5 and 6 are stained with carbol-fuchsin, eosin, and methylene blue.

Fig. 1. × 240. Lung of Fowl 108, which died 35 days after intravenous inoculation with 50 mg. of culture of an avian virus (A IV). The figure shows: (1) growth of the bacilli into solid clumps, particularly in the connective tissue septa; (2) an almost complete absence of tissue reaction. The fowl's lung is more resistant than the liver and spleen to the avian bacillus: but, after intravenous inoculation, lung tissue which appears normal to the naked eye may contain numerous bacilli.

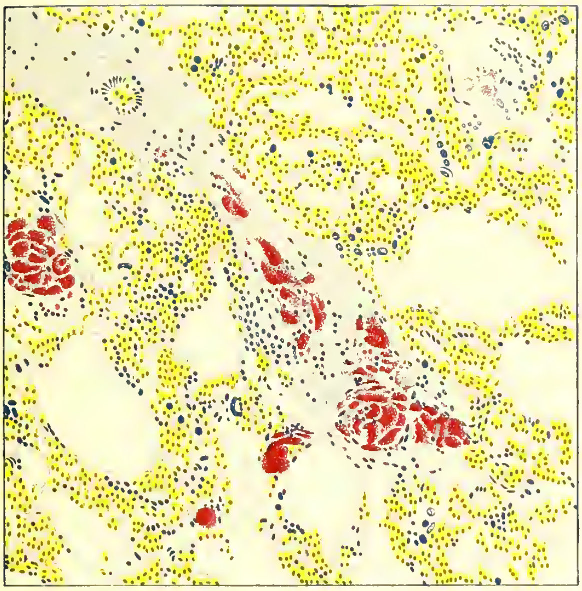
Fig. 2. × 240. Lung of Fowl 82, which died 53 days after intravenous inoculation with 10 mg. of culture of mammalian bacilli (from the bovine virus B XXVI). The specimen shows semi-caseous tubercles which are non-vascular and are bounded by a well marked fibrous zone. Within the interior of the tubercles the bacilli have evidently multiplied. (Such multiplication of mammalian bacilli in the tissues of the fowl is not very common.) Comparing Figs. 1 and 2, it is noteworthy that the mammalian bacillus has caused more tissue destruction of the fowl's lung than the avian bacillus.

Fig. 3. × 215. Spleen of Fowl 100, which died 33 days after intravenous inoculation with 1 mg. of culture of P III (a typical avian virus isolated from a pig). This is typical of the fowl's spleen after infection by the intravenous method with avian bacilli. Note:—(1) the abundant growth of bacilli into discrete islands; (2) the formation, round each group of bacilli, of concentric layers of hyaline deposit; (3) the presence, at the margin of some of the larger nodules, of a scanty layer of fibrous tissue; (4) the general absence of any tissue reaction leading to tubercle formation.

Fig. 4. × 215. Spleen of Fowl 26, killed 92 days after feeding with 10 mg. of culture of P XXI (a typical avian virus isolated from a pig). This lesion, in contrast to Fig. 3, is typical of the chronic type of disease produced in the fowl by avian bacilli not directly introduced into the blood-stream. Note:—(1) the broad peripheral zone of fibrous tissue; (2) internal to this, the zone of nuclear multiplication; (3) the completely caseous centre. In such lesions bacilli are usually scarce; none were seen in the section of the particular lesion illustrated. This scarcity of bacilli is to be contrasted with the abundant growth of bacilli after intravenous inoculation.

Fig. 5. × 294. Liver of Fowl 264, killed 162 days after feeding with the tissues of a rat which had been infected with an avian virus (A X). A portion of a typical chronic tubercle produced by feeding. Note:—(1) the marginal portion of the caseous centre is vacuolated, a feature which seems to be peculiar to areas of caseation produced by the avian bacillus; (2) the fibrous zone comes into close contact with the caseous area; (3) two bile ducts have survived in the outer part of the fibrous zone; (4) external to the fibrous zone is a dense infiltration of the fowl's ordinary "finely granular" leucocytes; (5) some red corpuscles have survived in the affected areas, though apparently cut off from the blood-channels; (6) bacilli are confined to the caseous centre, where they occur in small numbers.

Fig. 6. × 600. From the wall of a large lesion in the intestine of Fowl 264. Note:—(1) in the caseous area, to the right, several red corpuscles have survived but show nuclear disintegration, which makes them resemble mammalian leucocytes (this survival of the fowl's red corpuscles within caseous areas is common); (2) in the cellular wall there is an abundant proliferation and nuclear fragmentation of cells of endothelial type; (3) penetrating this layer is an infiltration of the fowl's multinuclear leucocytes; (4) the granules of these leucocytes are somewhat oblong; (5) bacilli are not confined to the caseous centre; there is one in the wall of the lesion at the extreme left of the figure.



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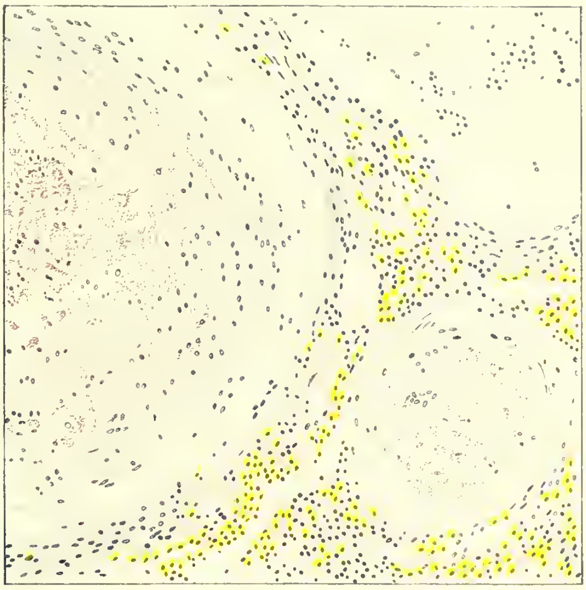

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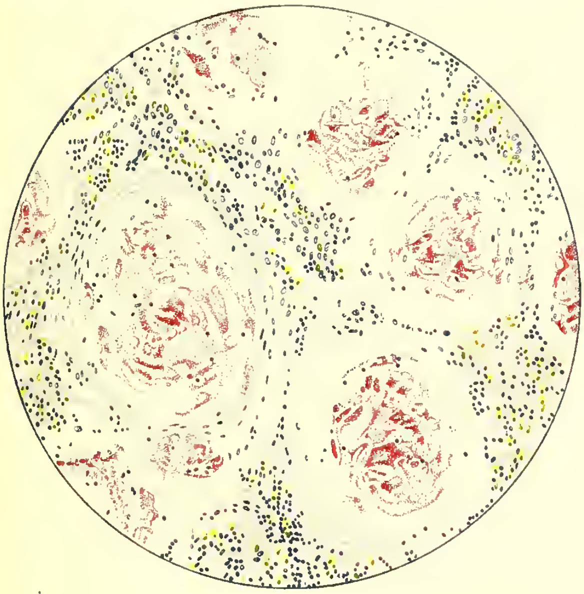

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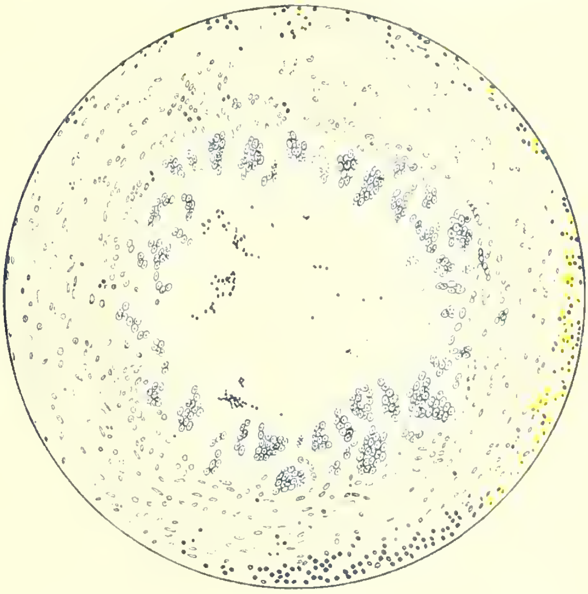

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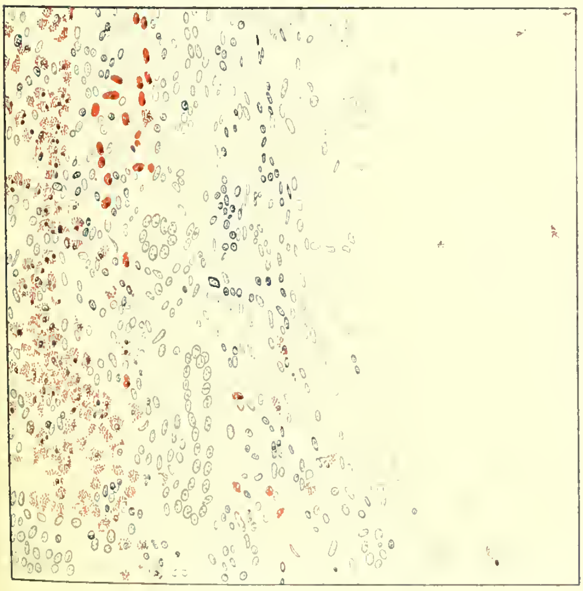

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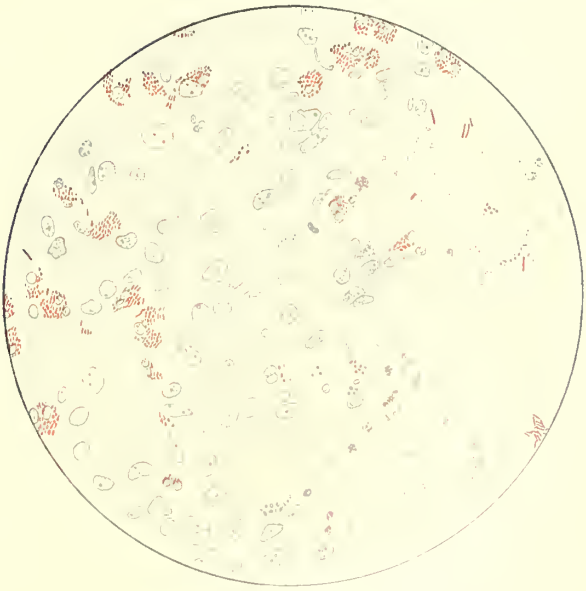

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J.R Ford del.

PLATE XIII.

STAIN.—Carbol-fuchsin followed (1) by methylene blue for Figs. 1, 3, and 5, (2) by orange G and methylene blue for Fig. 2, and (3) by eosin and methylene blue for Figs. 4, 6, and 7.

Fig. 1. × 77. Lung of Fowl 158, which died 108 days after subcutaneous inoculation with 1 mg. of culture of P II (a typical avian virus isolated from a pig). An example showing that subcutaneous inoculation of avian bacilli, which causes a more chronic type of infection than intravenous inoculation, produces extensive patches of caseation in the lungs. (Contrast Fig. 1, Plate XII.) Comparing the smaller with the larger foci it will be seen that the bacilli grow for a time without producing any notable tissue reaction, then after a certain stage is reached caseation sets in. Note the absence of a zone of inflammatory reaction at the margin of the lesions.

Fig. 2. × 215. Lung of Fowl 80, which was killed 102 days after intravenous inoculation with 1 mg. of culture of B XXVI (a mammalian virus of bovine origin). A portion of a chronic tubercle of a similar type to the one shown in Fig. 2, Plate XII, but in a more advanced stage. In spite of the small dose the bovine bacilli have, in this exceptional case, succeeded in multiplying abundantly. They appear to be completely cut off from the circulating fluids of the body. Note :—(1) the sudden transition between the caseous area and the surrounding cellular area ; (2) very few bacilli have survived in the latter area ; (3) this area is again bounded by a broad fibrous zone ; (4) the transition between the loose cellular zone and the fibrous zone is abrupt ; (5) both zones are completely avascular.

Fig. 3. × 275. Lung of Goat 48, killed when ill 83 days after subcutaneous inoculation with 50 mg. of culture of an avian virus (A X). A portion of a caseous tubercle, the caseous interior being to the lower half of the drawing. Note :—(1) the scarcity of bacilli in the caseous area ; (2) their abundance and typical rosette formations in the cellular zone ; (3) the marked vascularity of this latter zone. (This is an exceptional case ; it is not the common effect of subcutaneous inoculation of avian bacilli in the goat.)

Fig. 4. × 275. Portal gland of Calf 544, which died 33 days after intravenous inoculation with 10 mg. of culture of an avian virus (A VIII). The bacilli have grown very abundantly, with typical rosette formations, but have produced very little tissue destruction. The absence of irritant effects is remarkable. Only one finely granular oxyphil leucocyte is present in the entire field. (It is in the capillary at the upper part of the drawing.)

Fig. 5. × 150. Portal gland of Calf 412, killed 99 days after subcutaneous inoculation with 50 mg. of culture of P XXI (a typical avian virus isolated from a pig). The lesion is indistinguishable from lesions which might have been produced by mammalian bacilli. Usually very little disease is produced in the calf by subcutaneous inoculation with avian bacilli, and in this case the amount of disease was slight and mild in type. It is noteworthy that there is not much tissue reaction at the periphery of the lesion; the bacilli appear to be growing in fairly large numbers, both in the caseous interior and in the cellular margin, without producing any peripheral reaction of an inflammatory nature. A comparison between Figs. 4 and 5 shows the difference in effect between intravenous and subcutaneous inoculation.

Fig. 6. × 215. Liver of Fowl 96, which died 37 days after intravenous inoculation with 10 mg. of culture of an avian virus (A II). Typical acute infection of the fowl's liver after intravenous inoculation with avian bacilli. The liver cells are to a large extent replaced by colonies of bacilli. There is an absence of tubercle formation and of invasion with multinuclear leucocytes. Contrast Fig. 5, Plate XII.

Fig. 7. × 600. Liver of Fowl 338, which died 19 days after intravenous inoculation with 6 mg. of culture of mammalian bacilli from the bovine virus B XXVI. Compared with Fig. 6, this figure shows the difference in the action of mammalian bacilli upon the fowl's liver. The bacilli are scanty, and those which have survived have become long and somewhat beaded ; they show no signs of continued growth. The lesion shows necrotic change of the liver cells. There is no marked cellular infiltration. A few leucocytes and red blood-corpuscles are seen.

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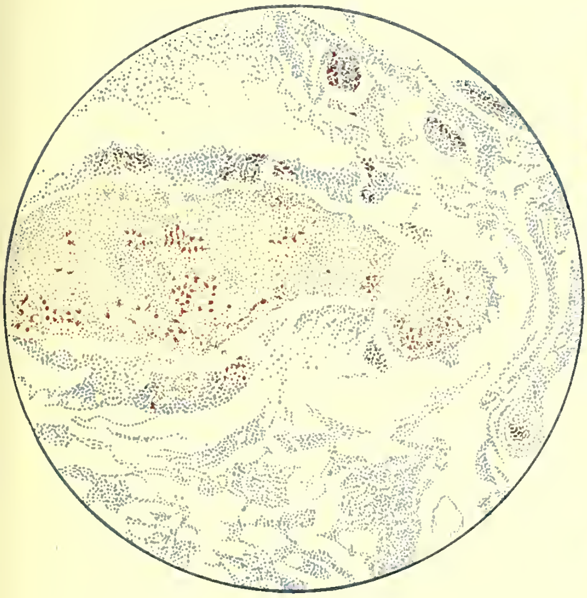
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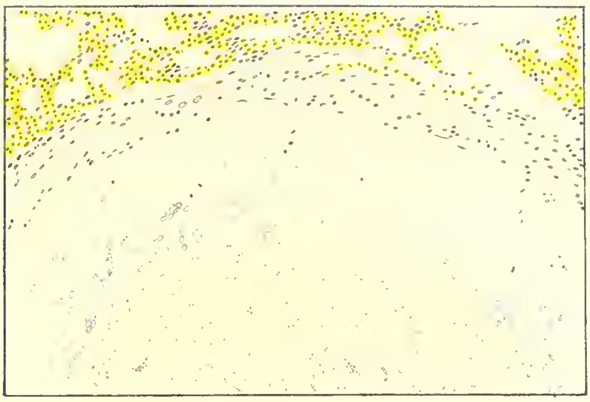
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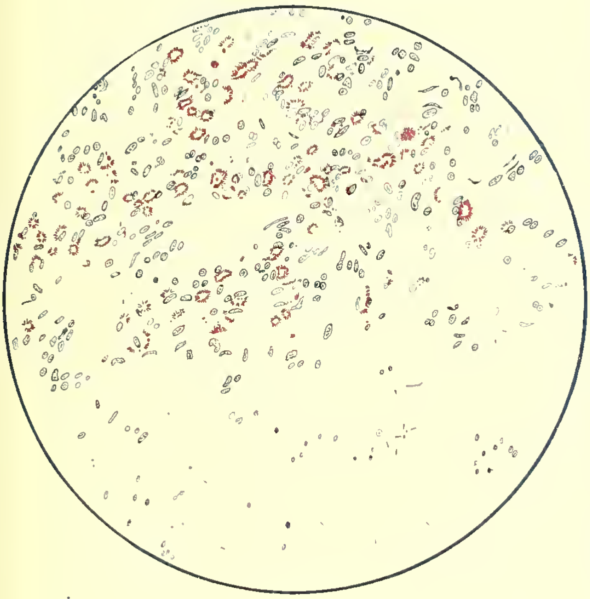
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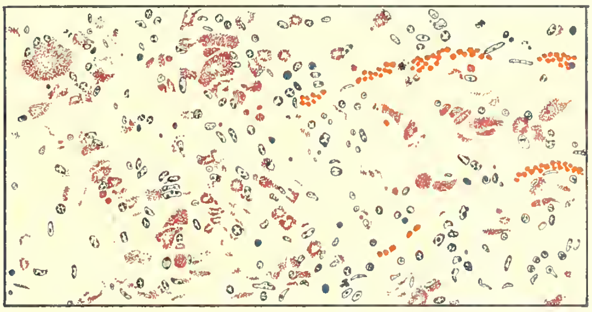
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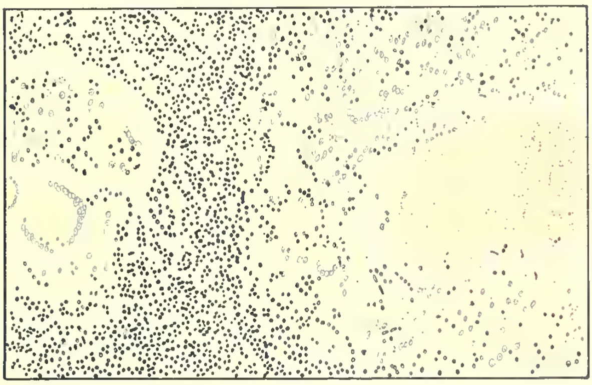
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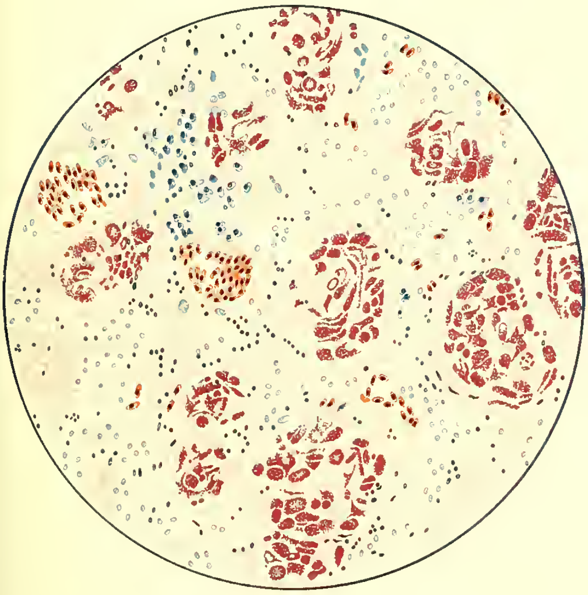
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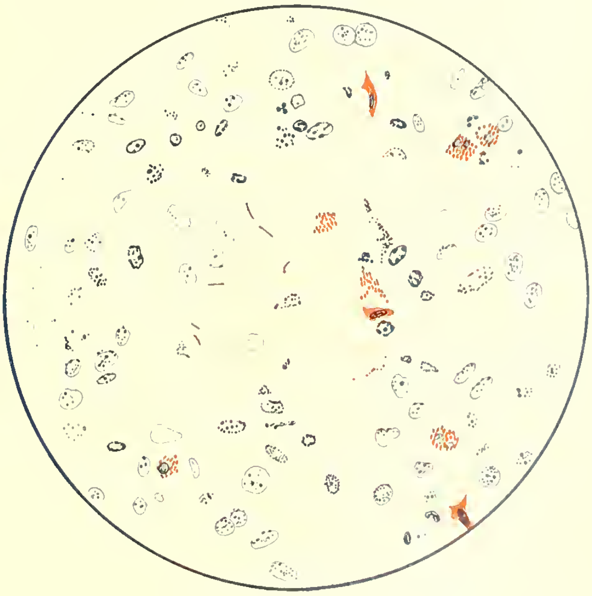
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J. R. Ford del.

Shiells & Ford, lith.

PLATE XIV.

STAIN.—For Figs. 1 and 2, carbol-fuchsin and methylene blue; for Figs. 3–6, carbol-fucshin, eosin, and methylene blue.

Fig. 1. × 275. Lung of Monkey 198, which died 28 days after intravenous inoculation with 100 mg. of culture of avian bacilli (A X). Numerous bacilli are present in the alveolar walls but have caused no tissue reaction. The specimen shows a remarkable absence of irritant or other pathological change after a large intravenous dose.

Fig. 2. × 275. Spleen of Monkey 198. Profuse growth of bacilli, with no marked tissue reaction.

Fig. 3. × 340. Lung of Pig 236, which died 120 days after intravenous inoculation with an emulsion of tissue (Calf 566) containing avian bacilli (A X). Profuse multiplication of bacilli, with rosette formations. Thickening of alveolar walls; infiltration of small lymphocytes but not of multinuclear leucocytes.

Fig. 4. × 294. Mesenteric gland of Pig 178, killed 101 days after feeding with 100 mg. of culture of avian bacilli (A VII). The lymphoid tissue is almost completely replaced by bacilli. Masses of bacilli are also circulating in the lymph sinus, a portion of which is shown in the upper part of the figure. Leucocytes are scanty; only two are present which stain with eosin. It is exceptional for this enormous growth of avian bacilli to occur in the pig after feeding.

Fig. 5. × 610. Liver of Pig 178. Profuse growth of bacilli and rosette formations, without tissue destruction. The interior of liver cells is seen to be the most favourable site for the growth of the bacilli. It will be noticed that several of the leucocytes contain bacilli. It is also interesting to note that bacilli occur within the one coarsely granular leucocyte present.

Fig. 6. × 240. Lung of Pig 178. Compared with the lung of Pig 236 (Fig. 3), where the bacilli arrived directly by intravenous inoculation of tissue emulsion, there is much less thickening of the alveolar walls. The bacilli are growing freely, and many are present within blood-vessels. There is an infiltration of small lymphocytes but not of multinuclear leucocytes.

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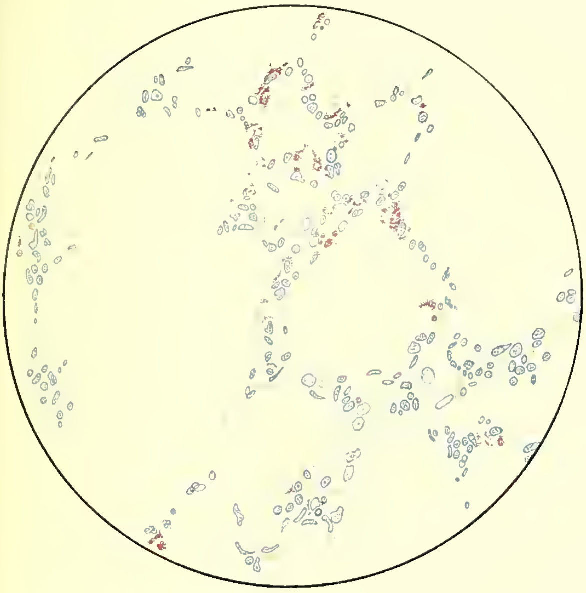
PLATE XIV.



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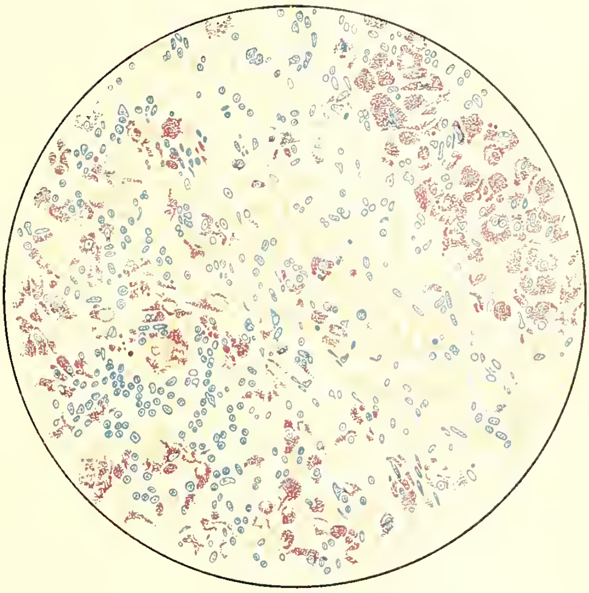

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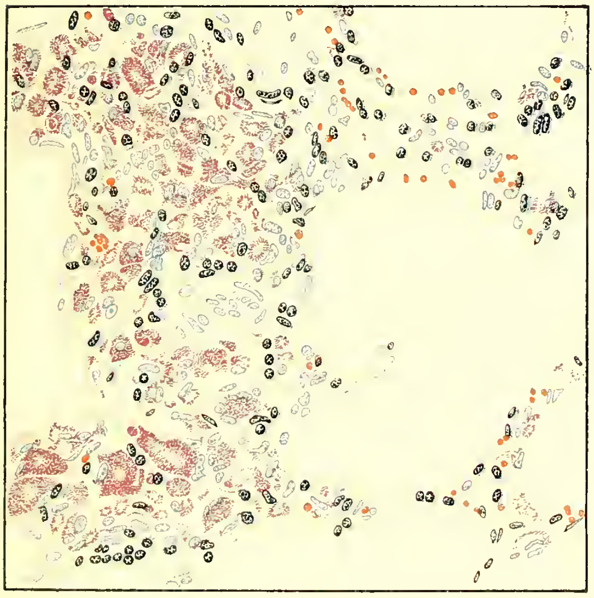

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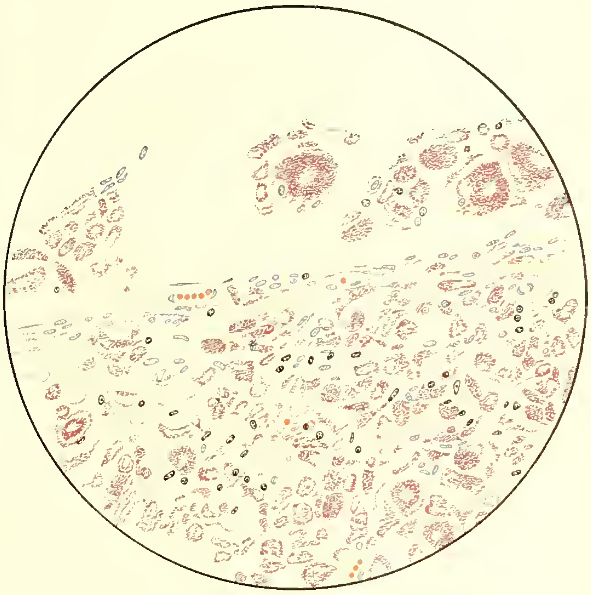

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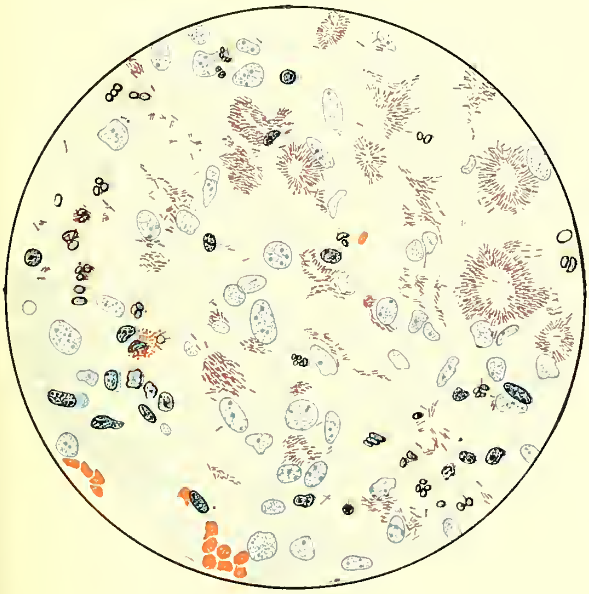

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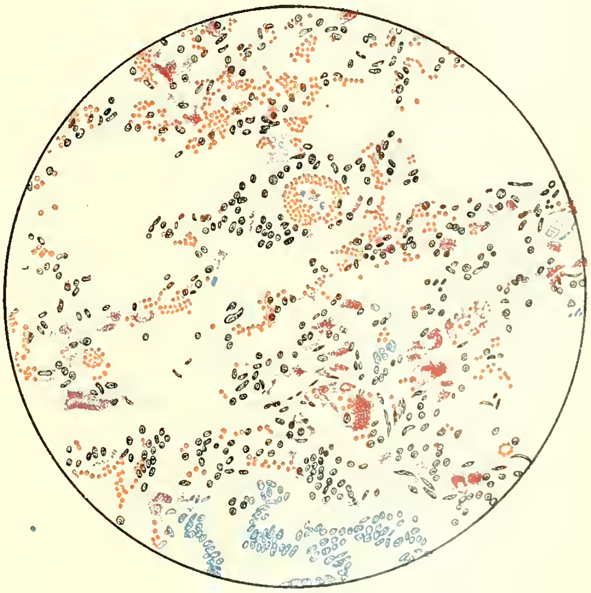

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J.R.Ford del.

Shiells & Ford, lith.

PLATE XV.

STAIN.—For Figs. 1, 2, 3, and 5, carbol-fuchsin, eosin, and methylene blue; for Figs. 4, 6, and 7, carbol-fuchsin and methylene blue.

Fig. 1. × 537. Lung of Calf 544, which died 33 days after intravenous inoculation with 10 mg. of culture of avian bacilli (A VIII). Compare Fig. 6 of Plate VIII, where the deposit of fibrin in the same lung is shown. The present figure shows an abundant catarrhal exudate, with well marked leucocytosis. None of the leucocytes contain bacilli.

Fig. 2. × 400. Lung of Rabbit 1556, killed 14 days after intravenous inoculation with 1 mg. of avian bacilli. (The liver of this rabbit is shown in Fig. 11, Plate XI.) After intravenous inoculation with avian bacilli, the lung of the rabbit is more resistant than that of the calf. This specimen shows a small patch of partial consolidation and commencing caseation. Compared with Fig. 1. there is less engorgement of blood-capillaries and less leucocytosis. None of the leucocytes present contain bacilli.

Fig. 3. × 275. Small intestine of Goat 48, killed 83 days after subcutaneous inoculation with 50 mg. of culture of avian bacilli (A X). The lumen of the gut is to the right. The epithelium and submucosa are extensively damaged and are overrun with bacilli, which have grown into rosettes. Multinuclear leucocytes are present, and some of them contain clumps of bacilli; the protoplasm of these cells has failed to stain with eosin.

Fig. 4. × 400. Synovial membrane of knee-joint of Rabbit 884, killed 143 days after subcutaneous inoculation with 10 mg. of culture of avian bacilli (A II). The specimen is typical of the affection of the joints which commonly occurs in rabbits in which a chronic type of disease has been set up by the avian bacillus.

Fig. 5. × 600. Lung of Guinea-pig 1962, killed 109 days after subcutaneous inoculation with avian bacilli (A V) contained in a tissue emulsion from a spontaneously affected pheasant. It is uncommon to find lesions produced in the lung of the guinea-pig by subcutaneous inoculation with avian bacilli. In this animal several small foci such as the one figured were present. There is disintegration of the epithelial and endothelial cells, with increase of their nuclei, and an infiltration of multinuclear leucocytes and lymphocytes. Bacilli are scanty; in the upper part of the field, to the left, two are present within a group of leucocytes.

Fig. 6. × 275. Liver of Monkey 196, which died 29 days after intravenous inoculation with 10 mg. of culture of an avian virus (A X). A tubercle produced by the avian bacillus in the endothelium of a blood-vessel. The lumen of the vessel lies to the upper part of the field.

Fig. 7. × 275. Spleen of Monkey 216, which died 66 days after subcutaneous inoculation with 1 mg. of culture of mammalian bacilli derived from an equine virus (E II). This specimen is figured because it bears a curious resemblance to infection with avian bacilli (dense multiplication of bacilli and growth into clumps, with little tissue change). Compare Fig. 2, Plate XIV. Culturally, however, the virus E II is typically mammalian.

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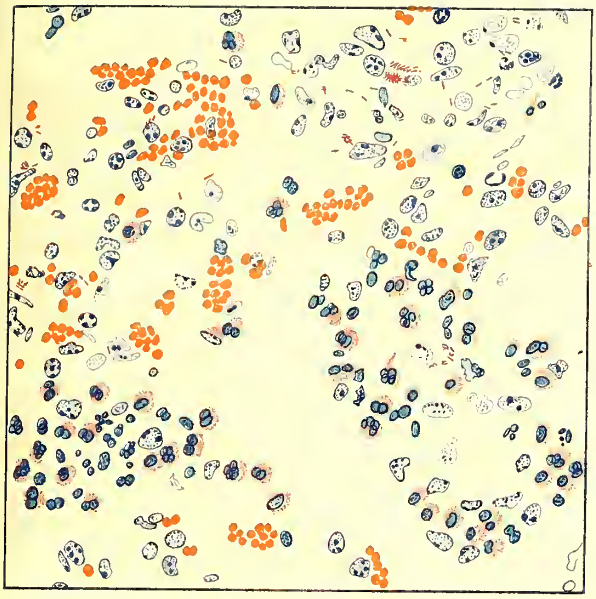
[illegible] 216, [illegible] [illegible] [illegible] [illegible] bacilli derived from [illegible] [illegible] [illegible] [illegible] [illegible] of bacilli and [illegible] [illegible] [illegible] [illegible] [illegible]

PLATE XV.



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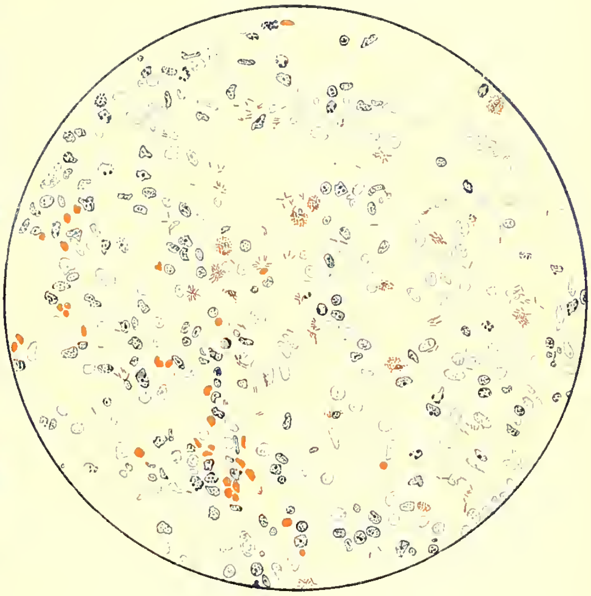

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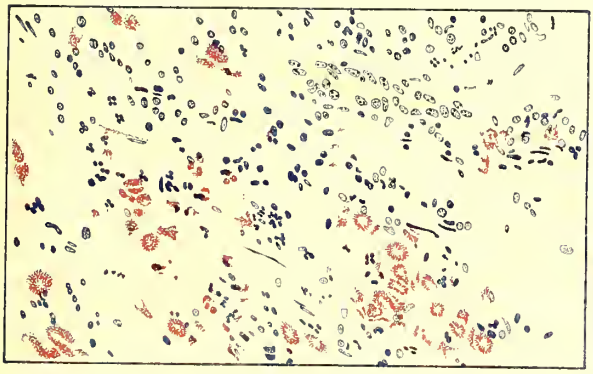

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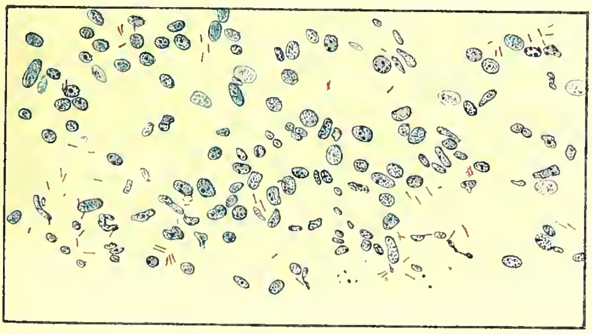

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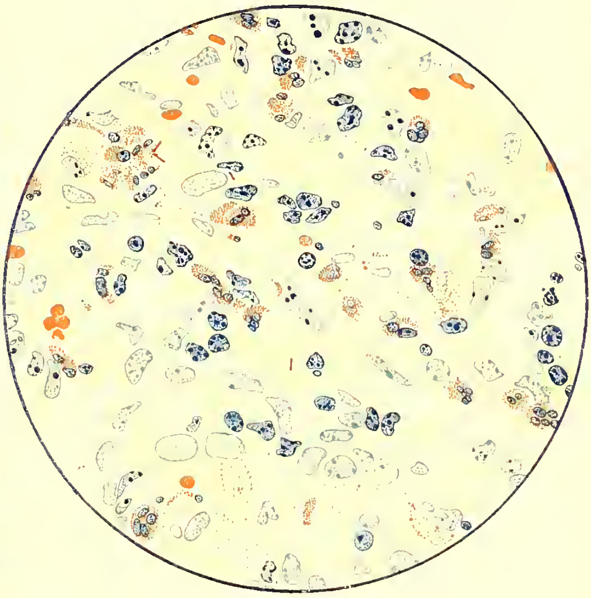

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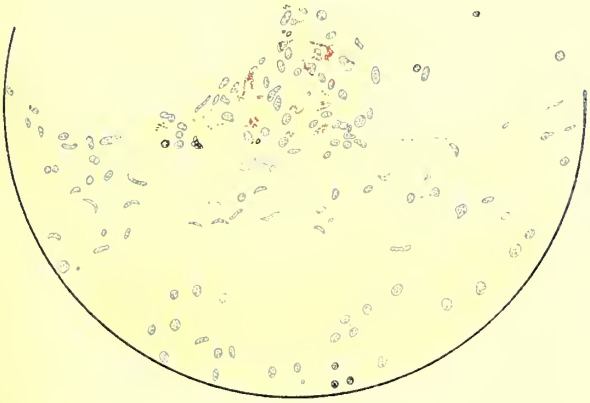

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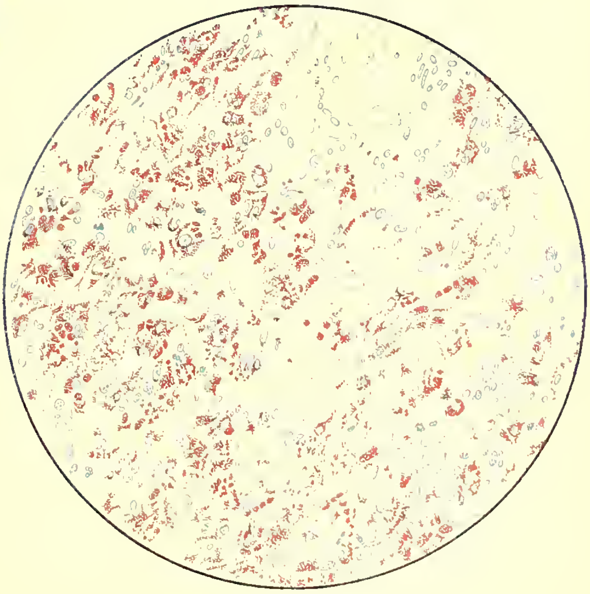

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PLATE XVI.

Stain.—Carbol-fuchsin.
Magnification.—× 2150.

Fig. 1. From a culture, 14 days old, on inspissated horse serum, of a typical mammalian virus of high pathogenicity to experimental animals and of poor cultural growth. The virus (P XXVI) was isolated from a pig and proved identical in every respect with numerous viruses isolated from cattle. The bacilli are short and comparatively thick; some of them show a central constriction.

Fig. 2. From a culture, 20 days old, on inspissated horse serum, of a mammalian virus of low experimental pathogenicity to bovines and rabbits and of vigorous cultural growth. The virus (H 23. J.P.) was isolated from a human being. The bacilli are very short; some of them show a central constriction. The clump at the bottom of the figure illustrates the tendency of mammalian bacilli to stick together and the difficulty of separating them by emulsification. The bacilli from serum cultures of this virus proved shorter than the average vigorously growing bacillus of human origin. This figure illustrates the impossibility of distinguishing with certainty, under the microscope, "human" from "bovine" bacilli.

Fig. 3. From a culture, 14 days old, on inspissated bovine serum, of a mammalian virus of low experimental pathogenicity to bovines and rabbits and of vigorous cultural growth. The virus (H 67. E.P.) was isolated from a human being. Many of the bacilli are exceptionally long and irregular for a young growth on pure serum. Short forms, some with a central constriction, are also present. The presence in a "human" virus of long, serum-grown forms such as those shown in this figure is by no means constant, and cannot be relied on as an aid to diagnosis.

Fig. 4. From a culture, 44 days old, on glycerinated broth, of a typical mammalian virus of high pathogenicity to experimental animals and of poor cultural growth. The virus (B I) was isolated from a bovine. The growth on broth was scanty. The bacilli show morphologically only a slight deviation from the serum-grown type.

Fig. 5. From a culture, 21 days old, on glycerinated broth, of a typical mammalian virus of high pathogenicity to experimental animals and of moderately good cultural growth. The virus (B V) was isolated from a bovine. The broth culture grew rapidly and in fair abundance. The long, irregular forms are very commonly met with in this type of broth culture, when obtained from bacilli of high experimental virulence.

Fig. 6. From a culture, 22 days old, on glycerinated broth, of a typical mammalian virus of low pathogenicity to experimental animals and of vigorous cultural growth. The virus (H 71. L.V.) was isolated from a human being. The bacilli, obtained from a copious growth, are for the most part long and curved, and with a tendency to beading. They show less irregularity than the bacilli from the more virulent virus shown in Fig. 5.

Fig. 7. From a culture, 44 days old, on glycerin-agar, of a mammalian virus (H 60. W.B.—Bronchial Gland) which was isolated from a human being. The occurrence of branched forms of the mammalian tubercle bacillus in cultures obtained from ordinary media, such as glycerinated agar, broth, or potato, is, in my experience, extremely rare. The figure also shows other forms of bacilli, some very long, some very short, and many with globular or oval, darkly stained bodies variously situated. Bacilli with such appearances as these are common in agar cultures.

Fig. 8. From a culture, 36 days old, on glycerinated potato, of a mammalian virus of high experimental virulence and fairly good cultural growth. The virus (B IX) was isolated from a bovine. The specimen shows the irregularity of morphology commonly exhibited by this type of bacillus when grown on potato. Compare Fig. 5 which shows a broth culture of a somewhat similar bacillus.

Fig. 9. From a culture, 2 months old, on glycerinated potato, of a mammalian virus of low experimental virulence and vigorous cultural growth. The virus (H 47. S.B.) was isolated from a human being. The specimen is characteristic of the varieties of morphology met with in a vigorously growing potato culture.

Figs. 10 and 11. From cultures, 2 months old, on glycerinated potato, of two mammalian viruses, P XII and H 49. T.C. (Calf 787). Examples of darkly stained, oval bodies, showing the development therefrom of new bacilli.

Fig. 12. From milk obtained from the udder of Cow 64, which had received an intramammary inoculation with a typical bovine virus (B I). The bacilli are of various lengths; many of them are curved and regularly beaded. The bacilli here shown are such as are commonly met with in cow's milk; it would obviously be impossible for anyone to decide, on morphological grounds, that they were of bovine rather than of human origin.

Fig. 13. From a smear made directly from human phthisical sputum. This virus (H 144) grew very luxuriantly in pure culture. The specimen illustrates morphological varieties of bacilli commonly met with in human sputum. It is to be noted that the short forms are common, as well as the longer forms. As in the case of Fig. 12, it would be impossible for anyone to decide, on morphological grounds, as to the human or bovine origin of the bacilli shown.

Fig. 14. From a culture, 16 days old, on inspissated glycerinated horse serum, of a typical avian virus (A VII). The bacilli are very small, many of them being no larger than cocci. The pointed extremities are a rather conspicuous feature, one extremity being frequently more sharply pointed than the other. There are notable differences in the intensity with which the bacilli are stained. The bacilli, unlike the sticky mammalian bacilli, readily distribute themselves evenly over the film.

Fig. 15. From a culture, 14 days old, on inspissated glycerinated bovine serum, of a typical avian virus (P III). The specimen shows that young, glycerinated serum cultures do not invariably consist of short forms. But the large and irregular forms here shown occur less frequently on serum than on agar or potato.

Fig. 16. From a culture, 2 months old, on glycerin-agar, of a typical avian virus (A X). In addition to short forms, long branching forms are found. The branching frequently emanates from a darkly stained spherical point, which in some instances is of a greater diameter than the breadth of the bacillus. Branching is much commoner with the avian than with the mammalian bacillus.

Fig. 17. From a culture, 42 days old, on glycerin-agar, of a typical avian virus (A III). In addition to short bacilli and coccal forms, there are bacilli of very large size, with club-shaped thickenings.

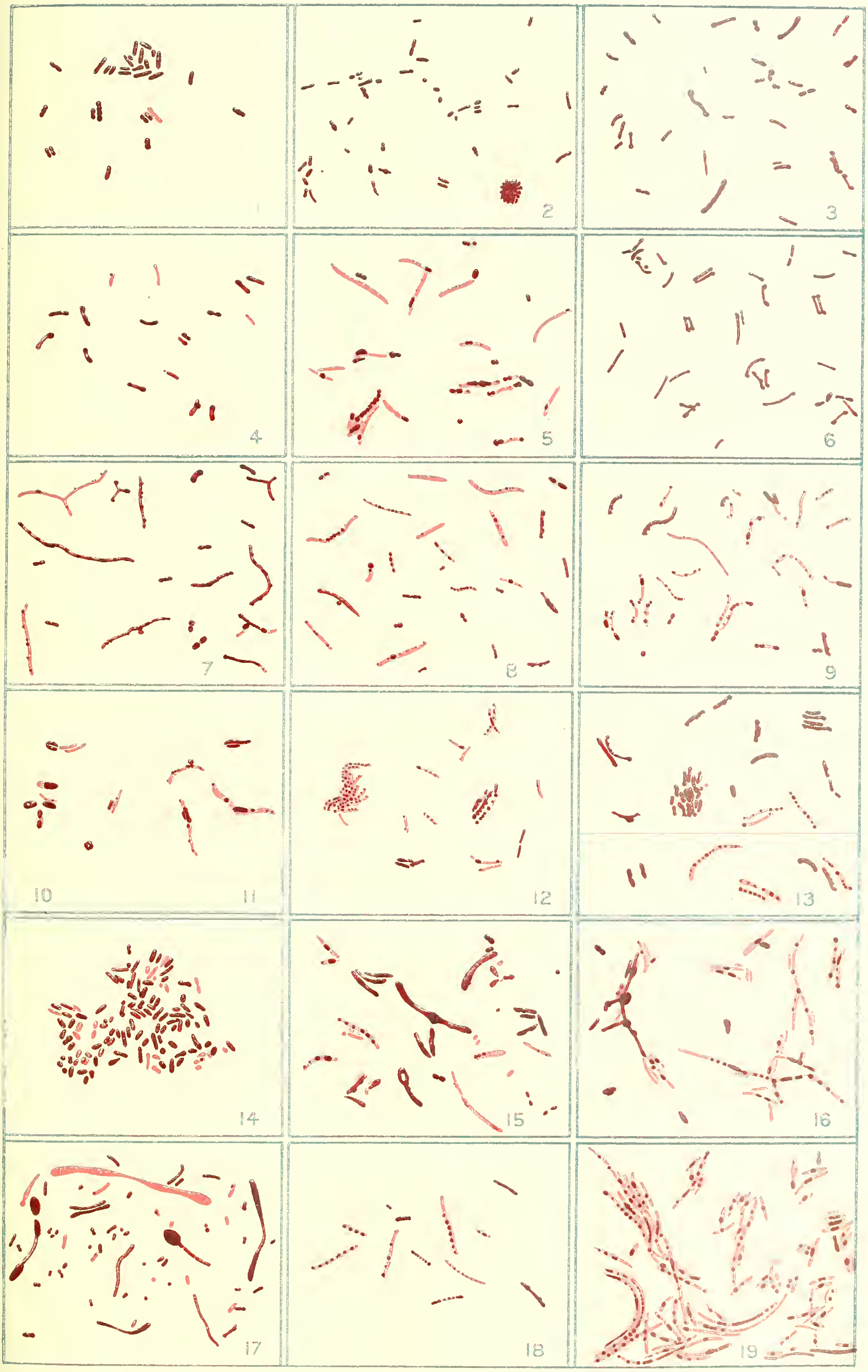
Fig. 18. From a culture, 2 months old, on glycerinated potato, of a typical avian virus (P XXVII). In this culture the bacilli show a tendency to regular beading, and are indistinguishable from mammalian bacilli. Compare Figs. 8 and 9.

Fig. 19. From a culture, 2 months old, on glycerinated potato, of a typical avian virus (A X). The bacilli show a tendency to grow in long, parallel threads.

PLATE XVI.

[illegible]

PLATE XVI



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REPORT ON THE RESULTS OF A CHEMICAL INVESTIGATION.

The following investigation was undertaken at the request of the Commission with the object of ascertaining whether it was possible by chemical means to differentiate between tubercle bacilli of the bovine and human types. Preliminary investigations having shown that the bacillus produced very extensive changes in the composition of the medium upon which it was grown, it was decided by the Commission that a systematic quantitative comparison of the action of the two types of bacilli should be made. (*See Final Report, Royal Commission on Tuberculosis, Page 8.*)

The actual experimental work was carried out at the Lister Institute during the years 1908–9, the various cultures being supplied from the Commission's laboratories at Stansted.

Mr. S. G. Walpole was appointed by the Commission as assistant, and conducted much of the experimental work under my supervision, and I have to express my thanks to him for the energy and zeal which he displayed throughout the course of the investigation, alike in the working out of the methods to be applied and in the systematic examination of a long series of cultures.

EXPERIMENTAL METHODS.

The Organisms Employed.

The various strains and viruses of the tubercle bacillus which were used in the experiments were supplied from the Stansted laboratories of the Commission, and were as follows. The designation used for the organism throughout this Appendix is placed in the first column, and a reference is given in each case to the complete account of the organism in the various appendices to the report.

I.—Bovine Tubercle Bacilli of Bovine Origin :—

B I	Virus B I (Cow 44). Vol. I. of Appendix to Second Interim Report, page 55.
B II	Virus B II (Heifer 66). Vol. I. of Appendix to Second Interim Report, page 111.
B IV	Virus B IV (Original material). Vol. I. of Appendix to Second Interim Report, page 161.
B IX	Virus B IX (Original material). Vol. I. of Appendix to Second Interim Report, page 245.
B X	Virus B X (Original material through G.P. 1070). Vol. I. of Appendix to Second Interim Report, page 259.

II.—Bovine Tubercle Bacilli of Porcine Origin :—

P XIV " O.M."	Virus P XIV (Original material through G.P. 1623). (Not fully virulent.) Vol. III. of Appendix to Final Report, pages 156, 240.
P XIV... ...	Virus P XIV (Calf 448). (Fully virulent.) Vol. III. of Appendix to Final Report, pages 156, 240.

III.—Avian Tubercle Bacillus of Porcine Origin :—

P II	Virus P II (Original material). Vol. III. of Appendix, page 154 ; Vol. IV. of Appendix, page 343.

IV.—Bovine Tubercle Bacilli of Human Origin :—

H 2	Virus H 2. " Sp. A." (Calf 93.) Vol. II. of Appendix to Second Interim Report, page 47.
H 19	Virus H 19 " S.W." (Goat 11.) Vol. II. of Appendix to Second Interim Report, page 469.
H 31	Virus H 31. " L.F." (Human cervical glands direct.) Vol. II. of Appendix to Second Interim Report, page 682.
H 90	Virus H 90. " I.P." (Human retroperitoneal gland direct.) Vol. I. of Appendix, page 134 ; Vol. III. of Appendix, page 18. Note.—The strain of culture obtained from the retroperitoneal gland used in these experiments grew luxuriantly on artificial media, like a human tubercle bacillus, but its virulence for bovines and rabbits was nearly equal to that of a bovine tubercle bacillus. This strain was subsequntly demonstrated to be a mixture of human and bovine tubercle bacilli. (*See* Vol. III. of the Appendix to the Final Report, page 18.)

V. – Human Tubercle Bacilli :—

H 12	Virus H 12. "H.N." (Calf 123.) Vol. II. of Appendix to Second Interim Report, page 259.
H 27	Virus H 27. "B.D." (Human cervical glands through G.P. 1216.) Vol. II. of Appendix to Second Interim Report, page 607.
H 49	Virus H 49. "T.C." (Human mesenteric glands direct.) Vol. III. of Appendix, pages 12, 317. NOTE.—The strains of culture obtained from this virus grew luxuriantly on artificial media, like a human tubercle bacillus, but their virulence for bovine animals was nearly equal to that of a bovine tubercle bacillus. After nine months artificial cultivations one of the strains (that known as the Blythwood strain, subsequently used for these experiments) was found to be reduced in virulence, behaving in all respects like a human tubercle bacillus. (*See* Vol. III. of Appendix to Final Report, page 12.) The other strain remained virulent, and was shown to be a mixture of human and bovine tubercle bacilli. (*See* Vol. III. of Appendix to Final Report, page 12.)
H 74	Virus H 74. "D.S." (Human bronchial gland through G.P. 2001.) Vol. I. of Appendix, page 116.
H 75	Virus H 75. "A.H." (Human peritoneal gland through G.P. 2018.) Vol. I. of Appendix, page 118.

GENERAL DESCRIPTION OF THE COURSE OF WORK.

Each strain of which the action on the medium was to be examined was inoculated into a series of three similar bottles of glycerol veal broth on the same date. These were incubated in the usual way at 37° for the desired intervals, after which they were removed and submitted to investigation. The bacilli were filtered off, dried and weighed, and an estimate was then made of the total volume of medium present. Aliquot portions of the filtrate were then taken and estimations made of the following factors :—

1. The total nitrogen.
2. Nitrogen non-precipitable by tannic acid.
3. Nitrogen non-precipitable by phosphotungstic acid.
4. Free and saline ammonia.
5. Ammonia liberated by hydrolysis with hydrochloric acid.
6. Acidity of the medium to phenolphthalein in the cold and at the boiling point.
7. The acids soluble in ether.
8. The amount of glycerol present.
9. The amount of phosphoric acid.

The comparison of the results obtained in the analyses of the original medium and of the medium after the growth upon it of the strain under investigation for different intervals of time yielded the desired information as to the nature of the changes produced in the medium and their dependence on the time of growth.

PREPARATION OF THE MEDIUM.

The glycerol veal broth employed for these experiments was prepared in the following manner. Lean veal, from which the fat had been removed, was minced and allowed to soak for four hours in saline tap-water, one litre of water and five grams of salt being taken for every 500 grams of meat. The whole was then heated to boiling, allowed to simmer for one hour and left over night. Next day it was again heated, the solid matter strained off, and after the addition of 10 grams per litre of Witte peptone and the requisite quantity of glycerol (usually 2·5 %) it was steamed for one hour. A sample was then taken and titrated by the method subsequently to be described (p. 7) and the calculated quantity of caustic soda added to bring the reaction of the finished broth to about + 15, so that one litre of finished medium required 15 cc. of normal caustic soda solution to make it neutral to phenolphthalein under the conditions of the titration test. The medium was then thoroughly well steamed for an hour, filtered through paper until quite bright and bottled.

TYPE AND ARRANGEMENT OF THE BOTTLES EMPLOYED.

The cultivation of the bacilli was conducted in green glass Winchester pint bottles of capacity about one litre. In order to diminish the loss of medium by evaporation, the neck of each bottle was closed by a rubber stopper, carrying a small glass tube which was loosely plugged with cotton wool. Exactly 400 cc. of the medium were measured into each bottle, previously thoroughly cleaned and dried, the stoppers were then inserted and the bottles heated for one hour in a steam steriliser on three consecutive days.

As a matter of practical necessity the medium was made up in a number of different batches, the amount prepared at one time varying from four to 16 litres. One or sometimes two bottles of each batch were analysed by the routine methods to determine the nature of the unattacked medium, such estimations being referred to as blank analyses.

It was observed that the broths prepared in this way on different occasions varied considerably in their nitrogen content. For the later batches therefore an empirical standard of nitrogen content was adopted according to which the finished medium should contain the nitrogen equivalent of 1000 cc. of decinormal ammonia per litre. This was attained by analysing the crude broth, and diluting to the requisite extent with 0·5 °/₀ salt solution, and finally bringing to the standard acidity (+ 15).

The following table shows the analytical results obtained for the various batches of medium employed. In most cases the blank analysis was carried out on a bottle which had been incubated alongside one of the inoculated bottles and this accounts for the fact that the volume of liquid is as a rule less than the 400 cc. which were invariably placed in each bottle. The numbers refer to the total amounts contained in this 400 cc., except those under the headings "acidity hot and cold," which give the titrations for 25 cc.

Number of Medium.	5.	6.	8.	8.	9.	9.	11.	12.	14.	15.
Vol. of liquid ; cc. ...	392	390·9	406·9	400	378·4	394	399·4	392·8	392·7	393·4
Specific gravity ...	1·018	1·021	1·034	1·029	1·020	1·022	1·030	1·023	1·019	1·019
Acidity, hot ; cc. N. acid	6·2	10·4	17·6	17·6	18·4	18·4	17·2	20	24·4	19·8
Acidity, cold	3·4	8·4	13·6	12·8	14	12·4	14	15·6	16	15·8
Total acidity, hot ...	24·4	40·7	70·8	70·4	69·6	72·4	68·7	78·6	97·6	79·2
Total Nitrogen; cc. N/10	648	1,086	1,518	1,501	1150	1185	1309	1263	1013	963·8
N. non-precipitable by tannic acid ; cc. N/10.	329	518	—	542	—	537	400	452·6	404·7	467·2
N. non-precipitable by phosphotungstic acid; cc. N/10.	136	147	166	159	—	160·6	130·6	155·1	113·5	119·2
Ammonia liberated by acid hydrolysis ; cc. N/10.	24·4	29	50·4	56	20·8	27·3	42·8	36·1	29·1	—
Free and saline ammonia ; cc. N/10.	34·1	40	46·7	45·2	33·3	46·7	40·7	37·7	31·4	29·9
Acids soluble in ether ; cc. N/10.	—	—	—	—	—	—	95·1	—	114	110·1
Glycerol ; grams. ...	7·59	7·16	7·50	7·60	—	8·27	20·36	12·38	7·98	7·38
Phosphate ; grams. P_2O_5	·1246	—	—	—	·4793	·4142	·2890	·4554		

Estimation of the Weight of Bacilli Produced and of the Volume of the Medium after Incubation.

After growth had continued for the requisite time the bottle was removed from the hot-room, allowed to cool and then weighed with the stopper and contents intact. The same operation, performed after the contents had been removed and the bottle cleaned and dried, gave the total weight of the contents (bacilli and fluid) present in the bottle after incubation (W). This was less than that of the contents present at the commencement of incubation, as a small but not inconsiderable evaporation always took place. The removal of the contents was performed before sterilisation by pouring the whole on to a tared filter cloth supported in a funnel. This cloth had previously been washed, dried in the air, and then in vacuo over sulphuric acid, and finally weighed. The clear filtrate was collected in a dry flask, and when the flow from the still moist bacilli had nearly ceased, the flask was removed and its contents reserved for analysis. Any small remainder of the pellicle was then washed from the bottle on to the cloth with distilled water and the washing was repeated four times. The funnel with cloth and bacilli in situ was then placed in the steam steriliser and steamed for one hour. After being steamed the cloth with its bacilli was spread out to dry at the room temperature for a day, then dried in vacuo over sulphuric acid for three days, and finally weighed between two watch glasses.

As it was impracticable to separate the filtrate from the bacilli quantitatively without dilution, an attempt had to be made to arrive at the total volume of the filtrate in another way. The weight of the bacilli as they exist in the medium was assumed to be three times that of the dry bacilli subsequently weighed after drying (B). This is an approximation based on general physiological experience and any error introduced into the final results of the analysis by the possible inaccuracy of this assumption is negligible, the total allowance to be made never exceeding about 4 cc.

The density of the filtrate (D) was then determined by means of a hydrometer. The volume of the medium (V) can then be calculated from the consideration that this equals the total weight of the contents of the bottle (W), less three times the weight of the dry bacilli and that the volume of this is given by dividing its weight by its density. Hence

$$V = (W-3B)/D.$$

Example. Analysis 104.

Weight of bottle on removal ...	1197·3 g.
Weight of empty bottle ...	798·5 g.
Weight of total contents (W) ...	398·8 g.
Weight of dry bacilli (B) ...	1·4 g.
Density (D)	1·017

Hence

$$V = \frac{398{\cdot}8 - 3 \times 1{\cdot}4}{1{\cdot}017} = 388 \text{ cc.}$$

Measured volumes of the filtrate were then taken for analysis; the volume, V, being known, these become aliquot parts, and the results obtained can readily be stated in terms of the total liquid medium.

ESTIMATION OF TOTAL NITROGEN.

For this purpose 25 cc. of the filtrate were subjected to the Kjeldhal process, and the result expressed in cc. of decinormal ammonia, all the usual corrections and precautions required to ensure accuracy being applied. The total nitrogen in the filtrate is $XV/25$ (where X = cc. of decinormal ammonia resulting from 25 cc. of the liquid).

ESTIMATION OF NITROGEN NON-PRECIPITABLE BY TANNIC ACID.

25 cc. of the medium were taken and added to an equal volume of tannic acid solution. The mixture was well shaken and allowed to stand for 24 hours in a corked flask. It was then filtered, 40 cc. of the filtrate taken, and the nitrogen estimated by Kjeldahl's process. A small correction is necessary for nitrogen present in the tannic acid.

The result is multiplied by 5/4, which gives the non-precipitable nitrogen in the 25 cc. taken, and from this is calculated the total in the whole volume of filtrate (V).

The tannic acid solution employed for precipitation had the composition—

Tannic acid, B.P.	7 g.
Glacial acetic acid	5 g.
Sodium chloride	6 g.
Distilled water, to	100 cc.

It is generally recognised that the amount of material precipitated by tannic acid from a mixture of digestion products of the proteins varies both with the concentration of the mixture and with the relative concentration of tannic acid and precipitable material. The results obtained by the use of tannic acid are therefore not absolute, but only comparative.

Since the action of the tubercle bacillus on the proteins of peptone beef broth produces a considerable amount of digestion, preliminary experiments were made to ascertain the effect of a variation in the amount of precipitable matter present. This was done by adding the same volume of tannic acid solution to varying quantities of the broth, all made up to the same volume, estimating the nitrogen in the filtrates as described above, and then calculating up in each case to 25 cc. Quantities of 10, 20, and 25 cc. of a broth containing 62 cc. of nitrogen (expressed as cc. of N/10 ammonia) per 25 cc., were made up in each case to 25 cc. with water and treated exactly as described above. The following were the results :—

	Kjeldahl.	Calculated for 25 cc. of broth.
1. L 10 cc. broth + 25 cc. tannic acid	9·8	30·6
2. 20 cc. ,, + 25 cc. ,, ,, ...	21·9	34·2
3. 25 cc. ,, + 25 cc. ,, ,, ...	27·9	34·9

It follows from this that the greater the excess of tannic acid the greater is the proportion of the nitrogen precipitated from beef broth peptone.

In all the experiments with tubercle bacilli the amount of non-precipitable nitrogen was found to increase during incubation so that the proportion precipitated after incubation would probably be greater than before incubation when the same volume of tannic acid was added. This would tend to diminish the non-precipitable fraction unduly, and the numbers obtained by this process are therefore probably affected in such a way that the amount of digestion appears to be less than it actually is.

NITROGEN NON-PRECIPITABLE BY PHOSPHOTUNGSTIC ACID.

25 cc. of the medium were placed in a graduated 75 cc. flask, along with 20 cc. of 20 per cent. sulphuric acid, and 5 cc. of phosphotungstic acid solution and the whole diluted to 75 cc., well shaken and allowed to stand for 24 hours. The liquid was then filtered through a dry paper, and 50 cc. taken and subjected to the Kjeldahl process. A correction of 0·1 cc. was required for the materials used.

The phosphotungstic acid solution employed contained 160 g. of Kahlbaum's phosphotungstic acid in 250 cc. of solution. Experiments to ascertain the effect of variation in the excess of phosphotungstic acid were made in the same manner as described under tannic acid. The results showed that the relations in this case were the inverse of those which exist in the case of tannic acid. The greater the excess of phosphotungstic acid, the less is the amount of nitrogen precipitated and the greater the apparent degree of digestion. This is probably due to the solubility of the phosphotungstic acid precipitate.

The following are the actual numbers obtained, the total volume being in each case 75 cc.

	Kjeldahl.	Calculated for 25 cc. broth.
1. 10 cc. peptone broth + 5 cc. phosphotungstic acid	2·07	5·18
2. 20 cc. ,, ,, + 5 cc. ,, ,,	3·42	4·28
3. 25 cc. ,, ,, + 5 cc. ,, ,,	3·97	3·97

The process of heating the phosphotungstic acid filtrate with sulphuric acid requires to be very carefully watched, as the liquid has a strong tendency to froth in the early stages and to "bump" in the later ones. It is best carried out by gently agitating the flask mechanically throughout the latter part of the operation.

ESTIMATION OF AMMONIA LIBERATED BY HYDROLYSIS WITH HYDROCHLORIC ACID.

In order to ascertain whether the amount of nitrogen present in such a form as to be liberated as ammonia by boiling normal hydrochloric acid might serve as a differential characteristic of various strains of tubercle bacilli the following method was adopted.

To 25 cc. of the medium were added 10 cc. of concentrated hydrochloric acid and the mixture was heated in a boiling water bath for 2 hours. The free and saline ammonia was then ascertained by distillation with magnesia (5 grams.) in the manner described below. The difference between the amounts of free ammonia before and after boiling with normal hydrochloric acid represents the amount liberated.

The chief sources of nitrogen liberated as ammonia in this way are the acid amides of the broth, which contains the group $\cdot CONH_2$. The method is in general use for the determination of nitrogen present in this form and was tested by estimations with asparagine, $CO(OH)\cdot CH_2\cdot CH(NH_2)\cdot CO\cdot NH_2$, which contains half of its nitrogen in the amide form. It was found, in agreement with the generally received idea, that about 98 per cent. of the amide nitrogen was split off as ammonia by boiling with normal hydrochloric acid for two hours. Thus a solution of asparagine which yielded 32·15 cc. of decinormal ammonia by Kjeldahl's process gave 15·7 cc. of free ammonia after boiling with hydrochloric acid, i.e. 15·7/16·07 or 97 per cent. of the theoretically possible amount.

ESTIMATION OF FREE AND SALINE AMMONIA.

The method adopted was that which consists in distilling the mixture containing proteins, amides, &c., with magnesia at 37°, receiving the ammonia evolved in standard acid and titrating in the usual way.

A large distillation flask with a rubber stopper carrying a tap is immersed in a water bath at 37° and is connected by rubber with a receiver, which consists of a filter flask carrying a pipette and containing the measured quantity of standard N/10 acid. The sample in which the free and saline ammonia is to be determined is introduced into the flask together with three grams (an excess) of magnesia (MgO) and the receiver connected to a good water pump. The pressure is lowered to about 15 mm. of mercury and the receiver is well cooled by a stream of tap-water. The distillation is then continued until the contents of the flask are brought to dryness, the time required being about one hour. The acid from the receiver is then titrated and the ammonia thus found by difference.

The accuracy of the method as carried out in this apparatus is shown by the following figures. A solution of 1·069 g. of ammonium chloride in 200 cc. of solution was employed.

25 cc. of this distilled with caustic soda gave 25·3 cc. N/10 ammonia. 25 cc. distilled with magnesia gave 25·25 cc. 25 cc. + 25 cc. of 2 per cent. asparagine solution-magnesia gave 25·25 cc.

ESTIMATION OF ACIDITY AND DENSITY.

Two portions of 25 cc. were pipetted into a 50 cc. Nessler glass and the density taken by means of a hydrometer. Then by means of the wetted pipette, 25 cc. were removed and put back into the bottle. The remaining 25 cc. were poured into a 400 cc. conical flask. The hydrometer bulb was then washed, and the washings collected in the Nessler glass and made up to the 50 cc. mark. This quantity of 50 cc. was then added to the liquid in the conical flask, so that the latter contained 25 cc. of the medium diluted to 75 cc. 2 cc. of 1 per cent. phenolphthalein solution were then added and the titration performed :—

1. In the cold. The vessel was immersed in the boiling water bath for $\frac{1}{4}$ hour, and then cooled, and the titration carried out until a definite pink colour was produced.
2. In the hot. The vessel was again immersed in boiling water, titrated until the end point was attained, then reheated and again titrated until the end reaction remained permanent. In the later experiments the heating was effected by placing the flasks on an electric heater until the temperature of the liquid was exactly 90°, and titrating at this temperature.

The algebraic sum of the two acidities is the total acidity at 90°.

ESTIMATION OF ACIDS SOLUBLE IN ETHER.

The method employed for the estimation of the acids soluble in ether consisted in acidifying the broth with dilute sulphuric acid and extracting with ether in a continuous extraction apparatus. The ethereal extract was then evaporated, and the residue boiled with barium carbonate and water, and filtered. The amount of barium in the filtrate was then estimated by precipitation as sulphate, and from this the equivalent in cc. of N/10 acid was calculated.

Several difficulties arose in the course of this estimation. In practice it was found that strictly accurate results could not be attained, as small amounts of acid continued to be extracted for a very long period. The presence of sodium chloride in the culture medium rendered it necessary to adopt some means of preventing hydrochloric acid being extracted along with the organic acids, and this was accomplished by adding a slight excess of silver sulphate to the medium before extraction. As finally adopted, the method only yielded comparative results, but as the object in view was to determine differences, it is probable that these were not affected by any serious error. 25 cc. of broth were placed in the extraction tube, and 0·25 g. of solid silver sulphate and 7·5 cc. of normal sulphuric acid added. The liquid was tested with congo-red paper to ascertain whether excess of mineral acid was present, and this was invariably found to be the case. The mixture was then shaken until the silver sulphate had reacted with the chloride, and was then extracted for 24 hours in a continuous extraction apparatus. A certain amount of frothing usually occurred, and it was necessary in those cases to evaporate the ether extract and again submit the residue dissolved in water to a second more rapid extraction for 12 hours.

The final extract was then evaporated on the water bath, 50 cc. of water added, and the whole heated with 1 g. of barium carbonate on the water bath for two hours. The liquid was then allowed to cool, filtered, and washed with cold water to about 70 cc. The filtrate was then heated to boiling, 1 cc. of dilute nitric acid added, and then 5 cc. of boiling dilute sulphuric acid. After digestion for an hour on the water bath, the barium sulphate was filtered off, washed, and weighed. From the weight thus found 0·0028 g. was subtracted to allow for the solubility of barium carbonate, this number having been obtained by a direct experiment.

Estimation of Glycerol.

The glycerol was invariably estimated by Zeisel and Fanto's method. This consists in heating the liquid with concentrated hydriodic acid, which converts the glycerol into isopropyl iodide. The latter is then passed into an alcoholic silver nitrate solution, where it is decomposed, forming silver iodide, the amount of which is estimated either gravimetrically or by titration.

The exact process employed was the following :—5 cc. of the broth, 15 cc. of Zeisel and Fanto's hydriodic acid (sp. gr. 1·9), and a small fragment of biscuit porcelain to prevent bumping, were placed in a small flask and heated at 137° in a glycerol bath, a stream of carbon dioxide, from a bottle of the compressed gas, being passed slowly through the apparatus. The flask was connected by ground glass joints with a wash bottle containing water and some red phosphorus to arrest iodine and this again with the absorption flask containing 30 cc. of standard alcoholic silver nitrate solution, approximately N/10. At the close of the operation, when no more precipitate formed in the absorption flask, the latter was disconnected and its contents made to 200 cc. with water. A portion of this was filtered through a dry paper and 50 cc. of the clear filtrate were titrated with standard potassium thiocyanate solution, a nitric acid solution of iron alum being used as indicator. The solutions were standardised by titration against N/10 sodium chloride solution prepared from pure dry sodium chloride. Owing to the fact that so small a volume of the medium has to be employed the accuracy of the method is not very great ; the error probably amounts to about 2–3 per cent. of the total amount of glycerol present.

Estimation of Phosphoric Acid.

25 cc. of the medium were heated in a Kjeldahl flask with 10 cc. of concentrated sulphuric acid and 20 cc. of concentrated nitric acid until all organic matter had been destroyed, a second addition of nitric acid being made if necessary (Neumann's method). After cooling the liquid was made to 100 cc. filtered and 90 cc. of the filtrate taken. This was made strongly alkaline with ammonia and cooled ; 15 cc. of magnesium citrate mixture were then added, the whole allowed to stand for 24 hours and the precipitated magnesium ammonium phosphate filtered off and weighed in the usual manner. The results are expressed as grams of phosphoric oxide (P_2O_5) per 400 cc. of medium.

ESTIMATION OF THE CHANGE OF ACIDITY PRODUCED BY THE ACTION OF PEPSIN AND PANCREATIN ON GLYCEROL BEEF BROTH.

In these experiments culture medium 14 was used. Solutions containing the requisite enzyme preparations were made up and added to the medium, a duplicate experiment being made in each case with a solution containing the enzyme rendered inactive by heat. The acidity was determined in each solution before and after incubation at 37° in presence of toluene. As the pepsin digestion was carried out in acid solution and the pancreatin experiment in presence of sodium carbonate, it was necessary to add an exactly equivalent amount of alkali or acid in each case before titrating. The method of titration adopted was as follows :—25 cc. of the solution to be titrated were mixed with 75 cc. of water and 2 cc. of a 1 per cent. solution of phenolphthalein in 50 per cent. alcohol, and maintained at 90° in an Erlenmeyer flask for 10 minutes, a stream of air being passed through to remove carbon dioxide. The flask was then cooled to room temperature and titrated until a definite pink tint was attained by the addition of N/10 potash. This gave the "cold titration" of the liquid. The flask was then again heated to 90° and the titration continued at this temperature until the end reaction had again been reached. The sum of the two titrations was taken as the "hot titration" and represented the acidity of the mixture.

1. Pepsin. The preparation used was the Glycerole of pepsin, concentrated, of Parke, Davis & Co. The solution exposed to digestion consisted of

475 cc. broth medium 14.
15 cc. pepsin solution (heated or unheated).
10 cc. of 10 per cent. hydrochloric acid.

The titration of the hydrochloric acid solution was 8·5 cc. N/10 potash, and hence this volume of potash was added to neutralise the hydrochloric acid to all the samples of peptic digestion, and the titration then conducted as described.

2. Pancreatin. Parke Davis' preparation was employed. The solution in this case consisted of—

2·5 grams of sodium carbonate.
475 cc. broth medium 14.
1 gram pancreatin (heated with water or unheated).
Water to 500 cc.

A duplicate sodium carbonate solution was made and titrated in the standard manner and required 22 cc. N/10 acid for 25 cc. This quantity of acid was therefore added to all samples of the pancreatin digestion before titration, and the process conducted as usual.

PART I.

THE CHANGES PRODUCED IN GLYCEROL BEEF BROTH BY THE CHEMICAL ACTION OF TUBERCLE BACILLI OF DIFFERENT ORIGINS.

I.—Weight of Bacilli obtained.

The following tables (I–III) exhibit the weights of bacilli obtained in 50 experiments, dealing with 16 Viruses and 17 strains. They are arranged in numerical order of the viruses, classified as Bovine Tubercle Bacilli of Bovine Origin, Bovine Tubercle Bacilli of Porcine Origin, Avian Tubercle Bacilli of Porcine Origin, Bovine Tubercle Bacilli of Human Origin and Human Tubercle Bacilli, and the tables show in each case the Virus employed, the medium used, the current number of the experiment, the time of incubation in days, the weight of dry culture obtained in grammes, and the average increase of weight in milligrammes per day of incubation. Table I contains the results obtained with 2 per cent. glycerol broth, Table II those obtained with 5 per cent. glycerol broth, and Table III the results of three experiments in which air was repeatedly passed over the culture during incubation.

Table I.

Weight of bacilli obtained on 2 per cent. glycerol broth.

Virus.	Medium.	Number.	Time of Incubation.	Weight of Culture.	Average Increase per day in Milligrammes.
Bovine Tubercle Bacilli of Bovine Origin.					
B I	5	12	38	0·45	11·8
		14	99	1·05	10·6
B II	8	74	142	0·839	5·91
B IV	5	9	34	0·845	24·9
		10	99	0·69	6·97
		11	149	0·59	3·96
B IX	14	95	54	0·73	13·5
		96	80	0·61	7·62
B X	14	92	96	1·29	14·3
		93	134	1·25	9·33
		90	210	2·09	9·99
Bovine Tubercle Bacilli of Porcine Origin.					
P XIV. "O.M." ...	14	104	78	1·43	18·3
		105	124	1·47	11·9
		106	155	1·56	10·1
P XIV	14	107	79	1·30	16·4
		108	124	1·20	9·68
		109	155	1·216	7·87
Avian Tubercle Bacilli of Porcine Origin.					
P II	14	101	99	0·70	7·0
		98	134	1·40	10·5
		100	222	0·88	3·97

BOVINE TUBERCLE BACILLI OF HUMAN ORIGIN.

Virus.	Medium.	Number.	Time of Incubation.	Weight of Culture.	Average Increase per day in Milligrammes.
H 2	12	87	114	1·81	17·1
		88	166	2·18	13·1
		89	232	2·20	9·48
H 19	12	85	93	1·18	12·7
		84	178	2·07	11·6
		83	254	1·44	5·67
H 31	8	60	116	1·33	11·5
H 90	15	119	27	0·64	23·7
		120	62	1·04	16·8

HUMAN TUBERCLE BACILLI.

Virus.	Medium.	Number.	Time of Incubation.	Weight of Culture.	Average Increase per day in Milligrammes.
H 12	6	22	35	0·23	6·57
		20	129	1·68	13·0
		21	198	1·59	8·03
H 27	6	25	35	0·42	12·0
		24	129	1·66	12·9
H 49	8	66	188	1·59	8·46
	9	53	105	2·20	21·0
		52	240	1·47	6·13
H 74	8	44	114	2·40	21·1
		43	200	1·10	5·5
		42	269	1·57	5·84
	15	117	26	0·35	13·5
		116	111	1·55	13·96
H 75	9	57	105	1·84	17·5
		56	240	1·29	5·38

TABLE II.

Weight of bacilli obtained on 5 per cent. glycerol broth.

Virus.	Medium.	Number.	Time of Incubation.	Weight of Culture.	Average Increase per day in Milligrammes.
B IV	11	70	133	0·77	5·79
		71	179	1·67	9·33
H 31	11	64	142	2·0	14·1

TABLE III.

Weight of bacilli obtained on glycerol broth in a current of air.

Virus.	Medium.	Number.	Time of Incubation.	Weight of Culture.	Average Increase per day in Milligrammes.
B X	14 glycerol 2 °/。	126	130	0·68	5·23
H 31	11 glycerol 5 °/。	76	145	1·57	10·8
H 74	15 glycerol 2 °/。	118	80	0·68	8·5

A comparison of the total weights of the bacilli obtained by cultivation under similar circumstances would serve to bring out any marked differences between the growing powers under those conditions of the various viruses and strains employed. In such a comparison the factor of time is of great importance. During the first period after growth commences the rate of increase of weight is comparatively rapid, but after a certain time this rate decreases very greatly and finally becomes zero. It is probable that autolysis proceeds concomitantly with growth throughout the whole period, but when growth ceases the autolysis continues and the weight of the culture may diminish.

In order to effect a strict comparison of this sort the media should be identical and the times of growth equal. In the experiments recorded in Table I it has been impossible rigidly to fulfil either of these conditions. The media were, however, all of the same kind and approximately of the same acidity and glycerol content, and the experiments were all conducted in precisely the same way.

As regards the influence of time, two data would be valuable; (*a*) the initial rate of growth, (*b*) the average yield obtainable. The determination of the initial rate of growth would require many more observations than have been made and is also rendered extremely difficult by the fact that after inoculation a considerable delay often occurs, followed by a very rapid growth.

In estimating the average yield obtainable it is necessary to omit, on the one hand, all experiments in which active growth was still taking place and, on the other hand, those in which active growth had long ceased and the weight of the culture was being steadily diminished by autolysis. It is impossible to fix these limits accurately and therefore an arbitrary limit has been assumed, only those weights being considered which were obtained by cultivation for 42 to 200 days.

In Table IV the viruses employed are arranged in the order of the average weights obtained within these limits, and they are divided into five arbitrary grades the limits of which were fixed by dividing the difference between the lowest and the highest yields into five equal parts.

For comparison a similar classification has been made (in Table V) showing the maximum yield obtained from each virus in any experiment and the two results are contrasted in Table VI, which shows the grades assigned on both these systems to the viruses arranged according to origin.

TABLE IV.

Classification of viruses by average weights obtained on 2 per cent. glycerol broth after incubation for 42–200 days.

Virus.	Number of Experiments.	Average.	Grade.	Limits of Grade.
B IV	2	0·64	I	0·64—0·91
B IX	2	0·67	I	
B II...	1	0·84	I	
H 90	1	1·04	II	0·91—1·18
B I	1	1·05	II	
P II	2	1·05	II	
P XIV	3	1·17	II	
B X	2	1·27	III	1·18—1·45
H 31	1	1·33	III	
P XIV. "O.M."	3	1·49	IV	1·45—1·72
H 19	2	1·62	IV	
H 12	2	1·64	IV	
H 27	1	1·66	IV	

TABLE IV—*continued.*

Virus.	Number of Experiments.	Average.	Grade.	Limits of Grade.
H 75	1	1·84	V	1·72—1·99
H 49	2	1·89	V	
H 74	2	1·97	V	
H 2	2	1·99	V	

TABLE V.

Classification of viruses by maximum weight obtained on 2 per cent. glycerol broth in any experiment.

Virus.	Weight.	Grade.	Limits of Grade.
B IV	0·69	I	0·69—1·032
B IX	0·73	I	
B II	0·84	I	
H 90	1·04	II	1·032—1·374
B I	1·05	II	
P XIV	1·30	II	
H 31	1·33	II	
P II	1·40	III	1·374—1·716
P XIV. "O.M."	1·56	III	
H 27	1·66	III	
H 12	1·68	III	
H 75	1·84	IV	1·716—2·058
H 19	2·07	V	2·058—2·40
B X	2·09	V	
H 2	2·20	V	
H 49	2·20	V	
H 74	2·40	V	

TABLE VI.

Grade and origin of viruses compared.

Origin and Nature.	Virus.	Grade.	
		Average.	Maximum.
Bovine, of Bovine Origin	**B I**	II	II
	B II	I	I
	B IV	I	I
	B IX	I	I
	B X	III	V

TABLE VI—*continued.*

Origin.	Virus.	Grade.	
		Average.	Maximum.
Bovine, of Porcine Origin	P XIV. "O.M."	IV	III
	P XIV	II	II
Avian, of Porcine Origin	P II	II	III
Bovine, of Human Origin	H 2	V	V
	H 19	IV	V
	H 31	III	II
	H 90	II	II
Human, of Human Origin... ...	H 12	IV	III
	H 27	IV	III
	H 49	V	V
	H 74	V	V
	H 75	V	IV

The general result of the classification by this method is that the bovine and avian viruses of bovine and porcine origin tend to be placed in the lower grades and the human viruses of human origin in the higher grades, whilst the bovine viruses of human origin are distributed throughout the system. Virus B X forms a marked exception to the other strains of bovine origin and is one of the best growing of the series.

II.—PROTEIN DIGESTION.

Several methods are available for estimating the general character and extent of the proteoclastic effect of the bacillus on the proteins of the culture medium, estimations having been made in each case of (1) the total nitrogen ; (2) the nitrogen non-precipitable by tannic acid ; (3) the nitrogen non-precipitable by phosphotungstic acid ; (4) the nitrogen liberated as ammonia by hydrolysis with normal hydrochloric acid ; (5) the free and saline ammonia. The results are contained in the following table and are in every case expressed in terms of cc. N/10 ammonia per 400 cc. of medium.

TABLE VII.

Changes in the nitrogenous constituents of glycerol beef broth produced by the tubercle bacillus.

No.	Virus.	Age.	Weight.	Change in					
				Tannic Precip.	Tannic Filtrate.	Total Nitrogen.	Phosphotungstic Filtrate.	Acid Hydrolysis.	Free Ammonia.
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
12	B I	38	0·45	−106·7	+ 52	− 54·7	+ 17	−24·4	+28·9
14		99	1·05	−117·5	+ 61·6	− 55·9	+ 21	−10·3	+30
74	B II	142	0·839	−181·7	+ 82	− 99·7	+ 92	−51·6	+41·9
9	B IV	34	0·845	−101·3	+ 12·6	− 88·7	+ 8·3	+ 1·2	− 3·7
10		99	0·69	−154·3	+105	− 49·3	+ 34	− 8·8	+33·9
11		149	0·59	−158·3	+118·8	− 39·5	+ 54·1	−14·2	+42·2
70	B IV	133	0·77	−333·1	+280	− 53·1	+198·4	− 2·4	+51
71		179	1·67	−349	+227	−122	+171·5	−41	−12·4
95	B IX	54	0·73	− 98·3	+ 54·3	− 44	+ 55·5	+ 0·7	− 6·4
96		80	0·61	− 65·3	+ 44·3	− 21	+ 53·5	+ 0·7	− 9·5

Table VII—*continued.*

No.	Virus.	Age.	Weight.	Change in					
1.	2.	3.	4.	Tannic Precip. 5.	Tannic Filtrate. 6.	Total Nitrogen. 7.	Phospho-tungstic Filtrate. 8.	Acid Hydrolysis. 9.	Free and Saline Ammonia. 10.
126*	B X	130	0·68	−191·1	+135·3	− 55·8	—	− 5·6	+32·9
92	B X	96	1·29	−165·1	+ 75·3	− 89·8	+ 57·5	− 2·7	− 1·1
93		134	1·25	−211	+126·3	− 84·7	+ 51	−19·8	+18·8
90		210	2·09	−211	+ 73	−138	+ 42·5	−10·8	+16·7
104	P XIV.	78	1·43	−166	+ 68·7	− 97·3	+ 54·1	−15·9	+13·6
105	" O.M."	124	1·47	−226·4	+117·9	−108·5	+ 68·3	−10·5	+ 7·5
106		155	1·56	−210·8	+100·6	−110·2	—	− 9	+13·4
107	P XIV	79	1·30	−200·6	+105·4	− 95·2	+ 82	− 5·1	+ 1·2
108		124	1·20	−221	+133·6	− 87·4	+ 93·3	−13·6	+15·1
109		155	1·22	−193·2	+117·2	− 76	—	− 7·5	+11·8
101	P II	99	0·7	− 84·3	+ 24·3	− 60	+ 51·5	−10·4	−29·1
98		134	1·4	−131·1	+ 37·5	− 93·6	+ 42·5	− 4·6	−28
100		222	0·88	−173·4	+112·7	− 60·7	+ 43	—	−11·9
22	H 12	35	0·23	− 23	+ 28	+ 5	+ 16·8	+16·7	− 5·7
20		129	1·68	− 97	+ 41	− 56	+ 67·3	− 4·9	−35·2
21		198	1·59	−126	+ 55	− 71	+ 90·4	− 3·4	−23
25	H 27	35	0·42	− 28	+ 24	− 4	+ 0·4	− 4·1	−10·4
24		129	1·66	−158	+ 43	−115	+ 41·3	− 4·7	−35·6
66	H 49	188	1·59	−138	+ 12	−126	+ 54	−14·1	+ 8·7
53	H 49	105	2·2	− 90	+ 20	− 70	+ 38	− 4	−30
52		240	1·47	−148	+ 92	− 56	+ 83	+ 2	− 1
44	H 74	114	2·4	−195·2	− 53·8	−249	− 6	− 6·5	−45·9
43		200	1·1	−181·7	+ 70·7	−111	+ 65	−19·5	−21
42		269	1·57	−159·4	+ 27·4	−132	+ 44·1	—	+46
117	H 74	26	0·35	− 41·6	− 14·5	− 56·1	− 0·3	—	−15·1
116		111	1·55	−157·3	+ 10·6	−146·7	+ 45·8	—	−22·1
118*	H 74	80	0·68	−116·8	+ 69·3	− 47·5	+ 98·9	—	− 0·97
57	H 75	105	1·84	−169	+ 96	− 73	+136	− 2·3	−19·7
56		240	1·29	−115·8	+ 79·8	− 36	+112	+14·8	+ 2·4
87	H 2	114	1·81	−154	+ 51	−103	+ 40·9	—	−29·9
88		166	2·18	−248·9	+ 64	−184·9	+ 34·5	−13·9	− 9·2
89		232	2·2	−210·2	+ 61·2	−149	+ 33	−18·5	+13·5
85	H 19	93	1·18	−211·5	+113	− 98·5	+ 44·9	−29·1	+19·3
84		178	2·07	−242·8	+ 93·8	−149	+ 57·9	−33	+18·6
83		254	1·44	−252·7	+157·7	− 95	+103·1	−15·2	+37·5
64	H 31	142	2·0	−371	+273	− 98	+166·4	−18·2	− 9·3
76*	H 31	145	1·57	−336	+221	−115	+ 90	−25·3	− 5·8
60	H 31	116	1·33	−207	+ 76	−131	+ 51·5	−26·6	−14·5
119	H 90	27	0·64	− 66·6	+ 1·8	− 64·8	+ 10·2	—	− 4·8
120		62	1·04	−105·4	+ 11·8	− 93·6	—	—	−24·9

* In experiments 126, 76, and 118 a current of air was frequently passed over the surface of the culture during incubation.

These numbers do not require much comment. They indicate that a vigorous digestion of the protein matter of the medium occurs, which as a general rule increases with the duration of incubation. This is rendered evident by the decrease in the nitrogen precipitable by tannic acid, the increase in that non-precipitable by tannic acid, the increase in that non-precipitable by phosphotungstic acid, and the decrease in the ammonia liberated by hydrolysis with hydrochloric acid.

The interpretation of the results is complicated in every case by the fact that after the growth of the bacillus the total nitrogen of the medium is less than that of the original medium, owing to assimilation by the organism and possibly other causes (p. 18). Hence, considering any factor which would be decreased by digestion, an observed decrease may really be due in part or entirely to assimilation, and hence represents a *maximum limit* to the extent of the digestion. On the other hand an observed increase in the factors which would tend to be increased by digestion represents a *minimum limit.*

(a) *Nitrogen precipitable by tannic acid* (*Col. 5*).—In every case this was found to have undergone a considerable decrease, amounting in some instances to as much as 40 per cent. of the original amount. The absolute amount digested as a rule increases with the period of incubation and the weight of the culture obtained.

(b) *Nitrogen non-precipitable by tannic acid* (*Col. 6*).—This factor also increases in every instance except two. If no assimilation or loss occurred, the increase in this factor would of course be equal to the decrease in the foregoing. Owing to assimilation by the organism, however, it is invariably less, by an amount equal to the difference between the original and final amounts of total nitrogen present in the liquid. Only in two instances (Nos. 44, 117) has assimilation been so much more rapid than the digestive process as to cause a diminution in this factor. The compounds which are not precipitated by tannic acid comprise ammonium salts, monaminoacids, hexone bases, and the lower polypeptides.

(c) *Nitrogen non-precipitable by phosphotungstic acid* (*Col. 8*).—The compounds coming under this heading are chiefly the aminoacids, and they are also increased in quantity by the action of the bacillus. This increase occurs at the expense both of the nitrogen precipitable and of that non-precipitable by tannic acid, compounds of the type of polypeptides, which are themselves precipitated by phosphotungstic acid, but not by tannic acid being converted by digestion into simpler substances which escape precipitation by either reagent. Hence this fraction may increase whilst the fraction not precipitated by tannic acid remains constant, or increases to a less degree, or even decreases. This is actually the case in many experiments (Nos. 57, 56, 21, 20, 101, etc.).

(d) *Nitrogen liberated as ammonia by hydrolysis with normal hydrochloric acid* (*Col. 9*).—This almost invariably decreases in amount, showing that the amides of the broth are either decomposed or assimilated to a greater or less extent.

(e) *Change in the amount of free and saline ammonia* (*Col. 10*).—The amount of free and saline ammonia, liberated at 37° in presence of magnesia, amounted in the original media to 18·5–46·7 cc. N/10 ammonia per 400 cc. of medium. The estimation was in every case carried out with 25 cc. of liquid, and was accurate to about 0·2 cc. so that the final number per 400 cc. should be accurate to about 3–4 cc.

The same remarks with regard to production and assimilation apply to this as to the other factors depending on protein decomposition, but with especial force, since the fact, that a decrease in the amount of ammonia present does occur in many cases, affords direct evidence that ammonia can supply, at all events in part, the nitrogen assimilated by the bacillus. Hence the change in the amount of free ammonia may be taken to indicate very clearly the relative preponderance of assimilation and production.

On this assumption it would be expected that different final conditions would be brought about according to the different weights of culture obtained and the times during which they were in contact with the liquid, a further factor being any specific difference between the various cultures.

Any exact quantitative comparison is rendered impossible by our ignorance of the exact time during which either assimilation or production of ammonia is in progress. The assimilation of nitrogen would be proportional to the weight of the culture obtained and the production probably proportional both to the mean weight of culture and the time of action, modified by the accumulation of the products of decomposition in the liquid. The change actually observed is the resultant of these two opposite tendencies.

That these considerations afford a complete *qualitative* explanation of the results observed is shown by an examination of the data. There are in all 17 series comprising 43 experiments, in which it is possible to compare the effect of the same virus on the same medium for different times. Out of these, 14 series (comprising 35 experiments) are completely consistent with the foregoing explanation, and a few of these are adduced to illustrate the prevailing conditions.

(*a*)	Number.	Age.	Weight.	Change in Free Ammonia.
	44	114	2·4	−45·9
	43	200	1·1	−21
	42	269	1·57	+46

Here the connection between a large growth and a large decrease in ammonia is very evident. The original amount of ammonia present in the medium was 46, so that the whole of this was assimilated (No. 44). The much smaller weight of culture obtained in experiment 43 has produced only half as great a diminution, the smaller amount of assimilation acting in the same direction as the longer period of incubation, whilst finally in experiment 42 the prolonged period of incubation of a larger weight of culture has resulted in an increase.

(*b*)	Number.	Age.	Weight.	Change in Ammonia.
	87	114	1·81	−29·9
	88	166	2·18	− 9·2
	89	232	2·2	+13·5

In this case the weights are not very different and the effect of increase of time of incubation is clearly seen in a progressive diminution in the decrease of free ammonia.

(*c*)	Number.	Age.	Weight.	Change in Ammonia.
	70	133	0·77	+51
	71	179	1·67	−12·4

In this case the assimilation due to the larger growth of bacillus has more than compensated for the increased time of incubation and has produced a decrease of 12·4 instead of an increase of 51.

The three series of experiments which are not entirely consistent with this mode of explanation are tabulated below.

TABLE VIII.

	Number.	Age.	Weight.	Change in Ammonia.	Change in Tannic Precipitate.
d	95	54	0·73	− 6·4	− 98·3
	96	80	0·61	− 9·5	− 65·3
e	104	78	1·43	+13·6	−166
	105	124	1·47	+ 7·5	−226·4
	106	155	1·56	+13·4	−210·8
f	107	79	1·30	+ 1·2	−200·6
	108	124	1·20	+15·1	−221
	109	155	1·22	+11·8	−193·2

(*d*) Here the smaller weight acting for a longer time has produced a slightly greater decrease in ammonia, whereas a smaller decrease would have been expected. The smaller amount of protein digestion (change in tannic precipitate) produced by No. 96 indicates that this culture was much less active than No. 95 in this respect, and this is probably the cause of the inconsistency.

(*e*) No. 104 has given an anomalous result, as it would have been expected to yield less ammonia than 105, especially as its digestive effect is much less. 105 and 106 are mutually consistent.

(*f*) The slight inconsistency between 108 and 109 is probably also due to the fact that the digestive power of 109 is less than that of 108.

It thus appears that out of 43 observations, only three are apparently qualitatively inconsistent with the explanation suggested, and of these only one (104) seems to be incapable of being brought into harmony with the others.

The comparison of the action of viruses of different origins is a matter of great difficulty. Dealing with the results statistically and including all the results obtained (50), the following table gives the numbers and percentages of the viruses of different origins which show no change, increase, and decrease respectively in the free and saline ammonia.

TABLE IX.

Nature of Virus.	Total Experiments.	No change.		Increase.		Decrease.	
		Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
Bovine, of Bovine Origin.	14	2	14·3	9	64·3	3	21·4
Bovine, of Porcine Origin.	6	1	16·7	5	83·3	0	0
Avian, of Porcine Origin.	3	0	0	0	0	3	100·0
Bovine, of Human Origin.	11	0	0	4	36·4	7	63·6
Human, of Human Origin.	16	3	18·75	2	12·5	11	68·5

A striking difference is at once manifest between the bovine viruses of bovine and porcine origin on the one hand and the bovine and human viruses of human origin on the other, the latter causing a decrease more frequently than an increase, whilst with the former the conditions are reversed. The single avian virus examined gave in all three experiments a marked decrease and thus agrees with the bovine and human viruses of human origin.

This state of things is qualitatively exactly what would be expected, without assuming any specific difference between the viruses of different origins, simply on account of the different weights of culture yielded by them (pp. 10–14).

It is, however, impossible to make a strictly quantitative comparison from this point of view, even between cultures grown on media of the same composition, because the rate and duration of growth, and the length of the period during which production of ammonia occurs are unknown.

(f) *Loss of nitrogen* (*Col. 7*).—The total nitrogen of the medium always decreases considerably during the growth of the bacillus, this being mainly due to the assimilation of nitrogen by the organism. The estimation of the nitrogen contained in the washed and dried bacilli usually, but not invariably, yields a number decidedly lower than that obtained by subtracting the final amount of nitrogen in the medium from its initial value. This difference appears to be a real one and not to be due to experimental error. It probably arises from the method adopted of treating the bacterial mass in order to obtain the weight of the culture. This consists in washing the bacilli four times with distilled water, then steaming them on the filter for one hour and finally drying them over sulphuric acid. There is every probability that some of the nitrogenous constituents of the cell would be removed in this way, leading to the observed discrepancy. Another minor cause of loss is perhaps to be found in the fact that in some cases the medium becomes alkaline to phenolphthaleïn. As a consequence of this alkalinity a certain loss of ammonia by evaporation is almost inevitable, and it must be remembered that, even when the final reaction is acid, it is quite possible that the medium has passed through an alkaline stage. No direct evidence of the loss of ammonia from the solution in this way has been obtained.

The following estimations show the extent of the discrepancy in question.

TABLE X.

Number.	Weight.	Per cent. of N.	N. in Dried and Washed Bacilli. (cc. of N/10 Ammonia.)	Loss of N from Medium. (cc. of N/10 Ammonia.)
88	2·18	8·05	125·4	184·9
85	1·18	7·63	64·3	98·5
60	1·33	7·34	69·8	131·0
66	1·59	8·34	94·7	126·0
70	0·77	8·51	46·8	53·1
74	0·84	8·50	51·0	99·7
92	1·29	7·36	67·8	89·8
64	2·0	7·11	101·7	98·0
87	1·81	8·27	106·9	103·0

III.—Change in Acidity.

The titrations were carried out on 25 cc. of the solution in the manner described on p. 7, and the results are expressed in cc. of N/10 acid per 400 cc. of medium. An error of 0·2 cc. in the titration, therefore, becomes 3·2 cc. in the total acidity, and this represents approximately the limit of accuracy attained.

In 41 out of 50 experiments the final acidity was lower than the original, whilst in the remaining nine experiments it was higher.

It is to be noted that in several cases the culture medium at the close of incubation was alkaline to phenolphthaleïn in the cold (after having been heated to 90° and then cooled), and in a few cases was also alkaline at 90°.

In the accompanying table (Table XI.) the actual acidity and the change in acidity are both given, together with the virus employed, age of culture, and original acidity of medium. All the results are expressed in ccs. of N/10 acid per 400 cc. of medium, and the acidities of those cultures which were alkaline in the cold are italicised. Increases in acidity are marked +, decreases –.

Table XI.

Change in Acidity.

Virus.	Number.	Age in Days.	Original Acidity of Medium.	Final Acidity.	Change in Acidity.
			Bovine, of Bovine Origin.		
B I	12	38	+ 24·4	+ 22	– 2·4
	14	99	”	– *14·1*	– 38·5
B II	74	142	+ 70·6	+ *19·2*	– 51·4
B IV	9	34	+ 24·4	+ *15·2*	– 9·2
	10	99	”	– *5·5*	– 29·9
	11	149	”	– *3·9*	– 28·3
	70†	133	+ 68·7	+ 33·6	– 35·1
	71†	199	”	+160	+ 91·3
B IX	95	54	+ 97·6	+ 36·8	– 60·8
	96	80	”	+ 46·4	– 51·2
B X	126*	130	+ 97·6	+ 43·2	– 54·4
	92	90	”	+ *40·8*	– 56·8
	93	134	”	+ 41·2	– 56·4
	90	210	”	+ 27·2	– 70·4
			Bovine, of Porcine Origin.		
P XIV. “O.M.” ...	104	78	+ 97·6	+ 25·6	– 72
	105	124	”	+ 24	– 73·6
	106	155	”	+ 27·2	– 70·4
P XIV	107	79	+ 97·6	+ 49·6	– 48
	108	124	”	+ 46·4	– 51·2
	109	155	”	+ 25·6	– 72
			Avian, of Porcine Origin.		
P II	101	99	+ 97·6	+ 42·4	– 55·2
	98	134	”	+ 25·6	– 72
	100	222	”	+ 34·4	– 63·2
			Human, of Human Origin.		
H 12	22	35	+ 40·7	+ 50·4	+ 9·7
	20	129	”	+ 4·8	– 35·9
	21	198	”	+ *57·0*	+ 16·3

* Air was frequently passed over the surface of this culture during incubation.

† Grown on 5 per cent. glycerol broth.

TABLE XI—*continued.*

HUMAN, OF HUMAN ORIGIN—*continued.*

Virus.	Number.	Age in Days.	Original Acidity of Medium.	Final Acidity.	Change in Acidity.
H 27	25	35	+ 40·7	+ 15·6	− 25·1
	24	129	”	+ 44·4	+ 3·7
H 49	66	188	+ 70·6	+ *30·4*	− 40·2
	53	105	+ 71	+ *6·4*	− 64·6
	52	240	”	+ 41·6	− 29·4
H 74	44	114	+ 70·6	+ *12·8*	− 57·8
	43	200	”	+150·4	+ 79·8
	42	269	”	+ 67·2	− 3·4
	117	26	+ 79·2	+ 60·8	− 18·4
	116	111	”	+ 16	− 63·2
	118†	80	”	+ *57·6*	− 21·4
H 75	57	105*	+ 71	+ *54* (?)	− 17·0
	56	240	”	+ 20·8	− 50·2

BOVINE, OF HUMAN ORIGIN.

Virus.	Number.	Age in Days.	Original Acidity of Medium.	Final Acidity.	Change in Acidity.
H 2	87	114	+ 78·6	+ 91	+ 12·4
	88	166	”	+ 85·6	+ 7
	89	232	”	+ 91·2	+ 12·6
H 19	85	93	+ 78·6	+102·4	+ 23·8
	84	178	”	+ 65·2	− 13·4
	83	254	”	+ 76·8	− 1·8
H 31	64‡	142	+ 68·7	+ 35·2	− 35·5
	76†	145	”	+ 64	− 4·7
	60	116	+ 70·6	+ 56	− 14·6
H 90	119	27	+ 79·2	+ 36	− 43·2
	120	62	”	+ 40	− 39·2

1. *Bovine viruses of bovine origin.*—Fourteen determinations were made with bovine viruses, and only in one case was an increase of acidity observed. This occurred in a culture of Virus B IV in 5 per cent. glycerol broth, 179 days old, and was so considerable as to lead to a suspicion of contamination. The material was, however, exhausted, and no further examination could be made. With this exception the results are in agreement with those of Theobald Smith and Griffith. The acidity is lowered to a certain point and then remains almost constant.

2. *Bovine viruses of porcine origin.*—In each of the six experiments, dealing with two viruses, the acidity was considerably lowered. As regards variation with time, the two series available for comparison differ somewhat. In the first case (Experiments 104, 105, 106) the decrease is almost constant for 78, 124, and 155 days. In the second series it increases with the duration of incubation (Experiments 107, 108, 109).

3. *Avian virus of porcine origin.*—In all three experiments decrease in acidity was observed; this was greater at 134 days than either at 99 or 122 days.

4. *Bovine viruses of human origin.*—A final decrease was observed in 7 cases out of 11, an increase in 4. Three series were available for observations on the variations of acidity with time. In the first of these (Experiments 87, 88, 89) there was a small increase at all times from 114 to 232 days. In the second (Experiments 85, 84, 83) an increase after 93 days was followed by a decrease at 178 and 254 days. In the third (Experiments 119, 120) there was an almost constant decrease both at 27 and 62 days.

5. *Human viruses.*—Out of 16 experiments, decrease in acidity was found in 12 cases, increase in 4. In five series it was found that the change of acidity

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* This solution was extremely dark-coloured and the reaction with phenolphthaleïn consequently uncertain.

† Air was frequently passed over the surface of these cultures during incubation.

‡ Grown on 5 per cent. glycerol broth.

followed the typical curve observed by Theobald Smith, a decrease in acidity being followed by an increase as the time of incubation was prolonged.

The factors involved in the change of acidity of glycerol broth.—The change in acidity of a complex medium, such as the glycerol broth employed in these experiments is a phenomenon which may involve a large number of factors, the observed change in acidity being the resultant of a number of different chemical changes, in which acid or alkali may be either produced or destroyed.

The chief of these factors in the case under consideration appear to be the following.

I. Production of acid.

(*a*) From glycerol, muscle sugar, or other non-nitrogenous constituents of the broth.

(*b*) By the elimination of ammonia from amino-acids and amides.

(*c*) By the proteoclastic decomposition of certain of the protein constituents of the broth.

II. Destruction of acid, by oxidation or assimilation of acids already existing in the broth, *e.g.*, lactic and phosphoric acids, or of acids produced by the processes mentioned under I.

III. Production of alkali, by the liberation of ammonia and hexone bases by the decomposition of proteins, amides, amino-acids, etc.

IV. Destruction of alkali, by assimilation of ammonia or other bases, both organic and inorganic, already existing in the broth, or of ammonia and bases produced as mentioned under III.

The analysis of the phenomena observed into these various factors has not been completely accomplished, but a certain amount of information on these points has been acquired and may be summarised under the same headings.

The fundamental observation of the whole series is the determination of the acidity (titration value) of the medium, and unfortunately the only practicable method available for this is not very satisfactory. Owing to the presence of very weak acids in the medium, the change of colour produced, even in the most sensitive indicators, is very gradual and this difficulty is often complicated by the colour of the liquid to be titrated. In addition to this the method employed, which only differs in some slight details from that used by Theobald Smith and Griffith, does not give absolutely reliable data for ascertaining the actual amount of acid produced or destroyed. In the first place a large amount of carbon dioxide is produced during the growth of the bacillus by the oxidation of the glycerol, etc. of the medium (p. 28). Some of this carbonic acid may remain combined in the form of carbonates or bicarbonates of inorganic bases, such as potash or magnesia, or of ammonia or other organic bases, and these carbonates entirely, or partially, escape decomposition when the liquid is heated to 90°. In this way a variable amount of carbon dioxide in the different experiments may be included as acid produced, or may mask the loss of acid, although it is not probable that this error would be large. In the second place it frequently happens that the liquid is alkaline to phenolphthaleïn after having been heated and cooled (*see* p. 19). Some of this alkalinity is no doubt due to the presence of free ammonia or ammonium carbonate, and in both cases loss occurs when the liquid is heated prior to titration, and the production of alkali (loss of acidity) appears too small. Hence the results obtained by taking the apparent change in acidity as a basis of calculation cannot be expected to be rigorously accurate. In spite of these considerations however, it has been thought advisable to prepare a table (Table XV) showing the residual change in acidity after allowing as far as possible for all the factors upon which observations have been made.

I. Production of acid.

(*a*) No direct evidence of the production of acid from glycerol, etc. has been obtained. That is to say (1) that no definite acid product of such a change has been isolated or detected in the medium after cultivation of the bacillus; (2) that there is no recognisable relation between the amounts of glycerol used and the production of acid (*see* table and discussion, p. 27).

(*b*) The liberation and assimilation of free ammonia, which is discussed on p. 16, provides a possible source of acid production, which may be illustrated by a simple example. The amino-acids, such as aminoacetic acid or glycocoll, $NH_2{\cdot}CH_2{\cdot}COOH$, are as a rule almost neutral to phenolphthaleïn, and in any case give a titration value much below their chemical equivalent. If these be decomposed into ammonia and the corresponding fatty acid or hydroxy-acid, for either action may occur, the result is that the ammonium salt of this acid is formed, and this is approximately neutral.

If now the ammonia be removed by assimilation, the free acid is left, which gives its full equivalent value when titrated. The reactions in question are illustrated by the following hypothetical equations dealing with aminoacetic acid.

(1) *a* $NH_2{\cdot}CH_2{\cdot}COOH + 2\,H = NH_3 + CH_3{\cdot}COOH = CH_3{\cdot}COONH_4.$
b $NH_2{\cdot}CH_2{\cdot}COOH + H_2O = NH_3 + HO{\cdot}CH_2{\cdot}COOH = HO{\cdot}CH_2.COONH_4.$

(2) *a* $CH_3{\cdot}COONH_4 = CH_3{\cdot}COOH + [NH_3].$
b $HO{\cdot}CH_2{\cdot}COONH_4 = HO{\cdot}CH_2{\cdot}COOH + [NH_3].$

The products of reaction (1) are neutral, those of reaction (2) acid.

Similar considerations hold with regard to the amides, such as asparagine, $COOH{\cdot}CH(NH_2){\cdot}CH_2{\cdot}CO{\cdot}NH_2$. The amido-group of this substance is readily removed by hydrolysis yielding ammonium aspartate,

$$COONH_4{\cdot}CH_2{\cdot}CH\ (NH_2){\cdot}COOH,$$

and this passes into aspartic acid if the ammonia be removed by assimilation. The titration value of these compounds are as follows, amounts equivalent to 10 cc. of a 0·5 °/₀ solution of asparagine being employed throughout.

	Acid equivalent.
10 cc. asparagine	2 cc. N/10 acid.
10 cc. ammonium aspartate	0·66 cc.
10 cc. aspartic acid	4 cc.

Hence the conversion of the asparagine into ammonium aspartate causes a diminution of acidity of 2 – 0·66 = 1·34 cc.; the removal of ammonia from this leaving aspartic acid, causes a rise of acidity of 4 – 0·66 = 3·34 cc., so that the nett result of the hydrolysis of the amide and the removal of ammonia is a rise in acidity of 4 – 2 = 2 cc.

No means has been found for the direct estimation of any acid produced in this way, and it would therefore appear as a part of the residual change in acidity, obtained by calculation from the observed change by allowing for the changes in ammonia and ether-soluble acids.

(*c*) The direct production of acid by the enzymatic hydrolysis of proteins has often been observed but varies very considerably with the nature of the protein and of the enzyme. Thus, Bayliss,* by the action of trypsin on casein, found that the solution rapidly became acid to litmus paper.

In order to obtain information as to the probable magnitude of this effect with glycerol beef broth, experiments were instituted with one of the media employed for the cultivation of the tubercle bacillus, which was subjected to digestion by pepsin and by pancreatin, the method being described on p. 9.

It is probable from analogy that the proteoclastic enzyme of the tubercle bacillus resembles trypsin more closely than pepsin in its action.

The results of the experiments were as follows, the figures for check experiments carried out with boiled solutions being also given, as well as the amount of nitrogen rendered precipitable by tannic acid, as an indication of the extent to which digestion proceeded. The results were actually obtained with 25 cc. of the medium and are expressed in terms of 400 cc. of the medium in uniformity with the tables throughout.

The total nitrogen in the case of the peptic digestion was 884·8 cc. N/10 ammonia, of which 497·6 were originally precipitable by tannic acid; in the case of the tryptic digestion the total was 999·2 cc., of which 558·4 were originally precipitable, in every case per 400 cc.

TABLE XII.

Time in Hours	Change in Acidity. CCs. N/10 Acid per 400 cc.			Nitrogen Rendered Non-precipitable by Tannic Acid. CCs. N/10 Ammonia.	
	Unboiled.	Boiled.	Difference.	Unboiled.	Boiled.
1. PEPTIC DIGESTION.					
39	+ 17·6	– 3·2	+ 20·8	—	—
216	+ 8·6	– 14·4	+ 23·0	137·6	0

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* Bayliss, "The Nature of Enzyme Action," p. 49. (London, 1908.)

TABLE XII—*continued.*

2. TRYPTIC DIGESTION.

Time in Hours.	Change in Acidity. CCs. N/10 Acid per 400 cc.			Nitrogen Rendered Non-precipitable by Tannic Acid. CCs. N/10 Ammonia.	
	Unboiled.	Boiled.	Difference.	Unboiled.	Boiled.
15	+ 25·8	− 3·2	+ 25·6	—	—
39	+ 73·6	+ 14·4	+ 59·2	—	—
216	+ 66·6	+ 19·2	+ 47·4	260·8	0

It will be observed that in both cases there is a decided increase in acidity and that the amount of digestion is of the same order of magnitude as the change produced by the tubercle bacillus (*see* table, p. 15).

Without pushing the analogy between the action of pancreatin or pepsin and that of the bacillus too far, the conclusion appears to be perfectly justified that the change in acidity due to the enzymatic hydrolysis of the proteins present through the agency of the bacillus is of the same order as the residual increase in acidity observed after all other determinable factors have been eliminated (*see* Table XV, Col. 8).

II. Destruction of acid.

This process has been traced in a certain number of the experiments, as regards (*a*) the acids soluble in ether, (*b*) phosphoric acid. No evidence has been obtained as to whether the acids not soluble in ether and the acids produced by the action of the bacillus on the medium are liable to similar changes. The assimilation of inorganic acids, other than phosphoric, appears only to occur to a very subordinate extent and has therefore a correspondingly small effect on the reaction of the medium.

(*a*) *Diminution in the amount of acids soluble in ether.*—A characteristic feature of the action of B. Tuberculosis on beef broth is the great change which is produced in the amount of acids soluble in ether. In all the 18 samples which have been examined from this point of view a large decrease in these acids has been observed. The results are embodied in the accompanying table XIII, in which the ether-soluble acids are expressed in ccs. of N/10 acid per 400 cc. of medium.

TABLE XIII.

Virus.	Number.	Age in Days.	Ether-Soluble Acids.			Decrease % of Original.
			Original in Medium.	Final.	Decrease.	
B IV	71	199	95·1	38·0	57·1	60·0
B IX	96	80	114	25·5	88·5	77·6
B X	92	90	114	19·7	94·3	82·7
	93	134	114	30·8	83·2	73·0
	*126	130	114	41·0	73·0	64·0
P II	101	99	114	7·2	106·8	93·7
	98	134	114	14·4	99·6	87·4
P XIV. "O.M." ...	104	78	114	19·4	94·6	83·0
	106	155	114	33·2	80·8	70·9
P XIV	107	79	114	21·7	92·3	81·0
	109	155	114	34·2	79·8	70·0
H 74	117	26	110·1	64·3	45·8	41·6
	116	111	110·1	33·5	76·6	69·6
	*118	80	110·1	23·3	86·8	78·8
H 90	119	27	110·1	46·8	63·3	57·5
	120	62	110·1	36·0	74·1	67·3
H 31	64	142	95·1	41·9	53·2	55·9
	*76	145	95·1	53·8	41·3	43·4

* In experiments 126, 118 and 76 air was repeatedly passed over the surface of the culture during incubation.

The decrease occurs in every case and varies in amount from 43 per cent. to 94 per cent. of the ether-soluble acids in the original medium. The action commences at an early stage in the growth of the organism and continues for a considerable time; thus with Virus H 74 (Experiments 117, 116), a decrease of 45·8 cc. (41·6 per cent.) occurred in 26 days, during which 0·35 gram. of bacillus was produced, whilst in 111 days it amounted to 76·6 cc. (69·6 per cent.), 1·55 grams of bacillus being produced. After a certain duration of incubation the amount present appears to remain stationary or even to increase slightly (Experiments 104, 106; 107, 109), but further observations would be required to establish the fact of such an increase. *This marked diminution in the amount of the ether-soluble acids must be regarded as one of the chief factors in the initial decrease in acidity of the medium, which occurs when tubercle bacilli of any origin are grown upon it.*

Nothing has been ascertained as to the nature of the reaction by which these acids are changed. It is to be noted, however, that a free supply of air (Experiments 76, 118, 126) does not appear to produce any increased effect.

(*b*) *Assimilation of phosphoric acid.*—A large amount of phosphorus enters into the composition of the tubercle bacillus, in the form of organic complexes, such as lecithides, nucleic acid, etc., and as a result of this the well-washed bacillus leaves an ash containing a high percentage of phosphoric pentoxide. The withdrawal of this oxide from the medium, in which it is almost entirely present in the form of mineral phosphate, must lead to an increase in the alkalinity (or decrease in the acidity) of the medium. The extent of this change is, however, only slight, as will be seen from the following numbers, obtained by estimating the percentage of phosphoric pentoxide in the washed and dried bacilli, and thus finding the amount withdrawn from the medium by the total weight of growth obtained. The removal of 0·1 gram of P_2O_5, present as neutral phosphate, diminishes the acidity by 28 cc. N/10 acid. As the maximum weight of growth obtained in any experiment was 2·4 gram., it will be seen that the maximum diminution due to this cause is not likely to exceed 14 or 15 cc. N/10 acid per 400 cc. of medium.

TABLE XIV.

Number.	Virus.	Weight of Growth.	Percentage of P_2O_5.	Total Weight of P_2O_5.	Consequent Fall in Total Acidity.
92	B X	1·29	2·60	0·034	9·5
70	B IV	0·77	2·16	0·017	4·7
96	B IX	0·61	2·34	0·014	4·0
64	H 31	2·0	2·34	0·047	13·2

The percentage of phosphoric pentoxide in the dried washed bacillus, which is dealt with in the second part of this report (p. 30), varies from about 1·2 -2·6 and hence the change of acidity due to this cause cannot be calculated accurately from the weight of growth obtained but must be ascertained by a special analysis of each crop of bacilli. The amount of phosphoric pentoxide removed from 400 cc. of the medium is so small that it cannot be accurately estimated by the examination of a small sample of the liquid and hence this estimation has not been made. The total amount of phosphoric pentoxide present in the media employed varied from 0·1236–0·4562 g. per 400 cc. Since the composition of the ash of the bacillus (de Schweinitz and Dorset) shows that basic oxides are present in the proportion of 2 RO : P_2O_5, which is also the proportion in which they form a neutral phosphate, it follows that the combined effect of the withdrawal of phosphoric oxide and of basic oxides, is to leave the acidity of the medium undisturbed. This condition however is not quite constant as the composition of the ash varies to some extent.

III and IV. Production and removal of alkali.

The production and assimilation of ammonia has already been fully discussed under the heading of protein decomposition (p. 16). Other basic products of hydrolysis are doubtless formed, which have an influence on the reaction of the medium. These would be for the most part contained in the fraction non-precipitable by tannic acid but precipitable by phospho-tungstic acid. As the basicity of this fraction after subtraction of ammonium salts, would be very variable no attempt has been made to bring the numbers expressing its amount into relation with the reaction of the medium, a further reason being that they would be highly inaccurate owing to the accumulation of the experimental error involved in the method of calculation.

As regards the inorganic bases, it has just been pointed out that these are present in the ash of the bacillus in approximately the ratio to phosphoric pentoxide required to form a neutral phosphate, and are therefore absorbed from the solution in the same ratio, so that it may be taken as a first approximation that the assimilation of phosphoric pentoxide and inorganic bases leaves the reaction of the medium undisturbed.

CALCULATION OF RESIDUAL CHANGE IN THE ACIDITY OF THE MEDIUM.

(*a*) *Influence of change in the amount of free and saline ammonia on the reaction of the medium.*—It is obvious that considered by itself the production of ammonia decreases the acidity or increases the alkalinity of the medium, whilst its removal by assimilation has the opposite effect. It follows that in order to ascertain the amount of acid produced from observations on the final acidity and on the change in the amount of ammonia, it is necessary to take the algebraic sum of the change in acidity, and the change in ammonia, production of acid and of ammonia being both reckoned positive, and diminution negative. Thus if the acidity is found diminished by 38, and the ammonia increased by exactly the same amount it is obvious that the acid destroyed is $-38 + 38 = 0$, the production of ammonia having caused the fall in acidity. In the same way if the acidity were to increase by 38 and the ammonia also to increase by the same amount, the production of acid would be $38 + 38 = 76$, enough acid having been produced to neutralise the 38 of ammonia and in addition to increase the acidity by 38. Finally, if the ammonia increased by 38 and the acidity remained unaltered, the production of acid would be 38. Such a case might arise by the conversion of a neutral aminoacid into its neutral ammonium salt, as explained on p. 22.

(*b*) *The influence of the removal of acids soluble in ether* may be allowed for in a similar manner. The removal of these acids makes the solution less acid, or more basic, and hence, in order to ascertain the residual amount of change in the acidity of the liquid after allowing for this it is necessary to subtract this number from the observed acidity algebraically, loss of acid being reckoned negative. Thus if the change in acidity is + 50 and the change in ether-soluble acids is − 30 it is obvious that there has been a production of acid of $50 - (-30) = 50 + 30 = 80$, sufficient acid having been produced to cover the loss of the 30 of ether-soluble acids and also to increase the final acidity by 50.

The number finally obtained, after allowing in this manner for the changes in the ammonia and ether-soluble acids, may be termed the *residual change in acidity*. If these were the only two changes which occurred it would be zero. Actually there is usually, but not invariably, a decided residual production of acid. Unfortunately the number thus obtained is not of a high order of accuracy, as it contains the accumulated errors of experiment of three sets of differences. It represents the resultant of the production and destruction of acid and alkali by all processes other than change in ammonia and ether-soluble acids. The chief factor in its composition is probably the increase in acidity due to digestion of the proteins, but production of acid from glycerol, etc., and changes in acidity due to change in the acids not soluble in ether, and to production of bases other than ammonia may also be represented. How far each of these contributes to the result can only be ascertained by further experiments.

In the following table are given the change in acidity, the change in ammonia, the nett acidity resulting from the algebraic addition of these two, the change in ether-soluble acids, and the residual acidity after subtracting this algebraically from the nett acidity.

TABLE XV.

Change in acidity as affected by change in ammonia and in acids soluble in ether.

Number. 1.	Virus. 2.	Age. 3.	Change in Acidity a. 4.	Change in Ammonia b. 5.	Nett Change in Acidity $a+b$. 6.	Change in Ether Soluble Acids c. 7.	Residual Production of Acid $a+b-c$. 8.
12	B I	38	− 2·4	+28·9	+ 26·5	—	—
14		99	−38·5	+30	− 8·5	—	—
74	B II	142	−51·4	+41·9	− 9·5	—	—
9	B IV	34	− 9·2	− 3·7	− 12·9	—	—
10		99	−29·9	+33·9	+ 4·0	—	—
11		149	−28·3	+42·2	+ 13·9	—	—

TABLE XV—*continued.*

Number. 1.	Virus. 2.	Age. 3.	Change in Acidity *a*. 4.	Change in Ammonia *b*. 5.	Nett Change in Acidity *a*+*b*. 6.	Change in Ether Soluble Acids *c*. 7.	Residual Production of Acidity *a*+*b*−*c*. 8.
70	B IV	133	−35·1	+51	+ 15·9	—	—
71		179	+91·3	−12·4	+ 78·9	− 57·1	+136
95	B IX	54	−60·8	− 6·4	− 67·2	—	—
96		80	−51·2	− 9·5	− 60·7	− 88·5	+ 27·8
*126	B X	130	−54·4	+32·9	− 21·5	− 73	+ 51·5
92	B X	96	−56·8	− 1·1	− 57·9	− 94·3	+ 36·6
93		134	−56·4	+18·8	− 37·6	− 83·2	+ 45·6
90		210	−70·4	+16·7	− 53·7	—	—
104	P XIV. “O.M.”	78	−72	+13·6	− 58·4	− 94·6	+ 36·2
105		124	−73·6	+ 7·5	− 66·1	—	—
106		155	−70·4	+13·4	− 57·0	− 80·8	+ 23·8
107	P XIV	79	−48	+ 1·2	− 46·8	− 92·3	+ 45·5
108		124	−51·2	+15·1	− 36·1	—	—
109		155	−72	+11·8	− 60·2	− 79·8	+ 19·6
101	P II	99	−55·2	−29·1	− 84·3	−106·8	+ 22·5
98		134	−72	−28	−100	− 99·6	− 0·4
100		222	−63·2	−11·9	− 75·1	—	—
22	H 12	35	+ 9·7	− 5·7	+ 4·0	—	—
20		129	−35·9	−35·2	− 71·1	—	—
21		198	+16·3	−23	− 6·7	—	—
25	H 27	35	−25·1	−10·4	− 35·5	—	—
24		129	+ 3·7	−35·6	− 31·9	—	—
66	H 49	188	−40·2	+ 8·7	− 31·5	—	—
53	H 49	105	−64·6	−30	− 94·6	—	—
52		240	−29·4	− 1	− 30·4	—	—
44	H 74	114	−57·8	−45·9	−103·7	—	—
43		200	+79·8	−21	+ 58·8	—	—
42		269	− 3·4	+46	+ 42·6	—	—
117	H 74	26	−18·4	−15·1	− 33·5	− 45·8	+ 12·3
116		111	−63·2	−22·1	− 85·3	− 76·6	− 8·7
*118	H 74	80	−21·4	− 0·97	− 22·4	− 86·8	+ 64·4
57	H 75	105	−17	−19·7	− 36·7	—	—
56		240	−50·2	+ 2·4	− 47·8	—	—
87	H 2	114	+12·4	−29·9	− 17·5	—	—
88		166	+ 7	− 9·2	− 2·2	—	—
89		232	+12·6	+13·5	+ 26·1	—	—
85	H 19	93	+23·8	+19·3	+ 43·1	—	—
84		178	−13·4	+18·6	+ 5·2	—	—
83		254	− 1·8	+37·5	+ 35·7	—	—
64	H 31	142	−35·5	− 9·3	− 44·8	− 53·2	+ 8·4
*76	H 31	145	− 4·7	− 5·8	− 10·5	− 41·3	+ 30·8
60	H 31	116	−14·6	−14·5	− 29·1	—	—
119	H 90	27	−43·2	− 4·8	− 48·0	− 63·3	+ 15·3
120		62	−29·2	−24·9	− 64·1	− 74·1	+ 10

IV.—CONSUMPTION OF GLYCEROL.

In the following table (XVI) the total weight of glycerol in the medium (Col. 5), the total weight of glycerol used (Col. 6), the amount used per gram. of culture obtained (Col. 7), and the amount used per gram. of culture per day of incubation (Col. 8), are given along with the age of the culture (Col. 3), the weight obtained (Col. 4), the change in acidity (Col. 9), the nett change in acidity after allowing for the change in free ammonia (Col. 10), and the residual acidity after allowing for the changes in both ammonia and ether-soluble acids (Col. 11).

The results are very irregular and appear to depend in large measure on the individual characteristics of the various growths. It is, however, noticeable that in the case of young cultures (less than 42 days), the total glycerol used and the weight used per gram. of bacillus obtained are both usually extremely low (Experiments 22. H 12 ; 25. H 27 ; 117. H 74 ; 119. H 90 ; and 9. B IV), there being only one exception to this rule (Experiment 12. B I). This appears to indicate that the glycerol is not extensively employed in the building up of the cell material of the bacillus, but is attacked after the actual growth of the cell has occurred.

No connection can be traced between the amount of glycerol used and the change in total acidity (Col. 8), and none is made evident by allowing for the production or disappearance of ammonia (Col. 9) and for the disappearance of acids soluble in ether (Col. 10).

TABLE XVI.

Relation of consumption of glycerol to weight of bacillus obtained and change in acidity.

No. 1.	Virus. 2	Age. 3.	Weight. 4.	Glycerol in Medium. 5.	Glycerol Used. 6.	Glycerol per Gram of Culture. 7.	Glycerol per Gram per Day. 8.	Change in Acidity. 9.	Nett Change in Acidity. 10.	Residual Change in Acidity. 11
12	**B I**	38	0·45	7·6	1·89	4·2	0·110	− 2·4	+26·5	—
14		99	1·05	7·6	5·2	4·9	0·050	−38·5	− 8·5	—
74	**B II**	142	0·839	7·55	3·5	4·2	0·029	−51·4	− 9·5	—
9	**B IV**	34	0·845	7·6	0·05	0·06	0·002	− 9·2	−12·9	—
10		99	0·69	7·6	2·20	3·2	0·032	−29·9	+ 4	—
11		149	0·59	7·6	2·26	3·8	0·026	−28·3	+13·9	—
70	**B IV**	133	0·77	20·36	1·86	2·4	0·018	−35·1	+15·9	—
71		179	1·67	20·36	8·86	5·3	0·029	+91·3	+78·9	+136
95	**B IX**	54	0·73	7·98	1·13	1·5	0·029	−60·8	−67·2	—
96		80	0·61	7·98	0·99	1·6	0·020	−51·2	−60·7	+27·8
*126	**B X**	130	0·68	7·98	1·11	1·6	0·012	−54·4	−21·5	—
92	**B X**	96	1·29	7·98	2·31	1·8	0·019	−56·8	−57·9	+36·6
93		134	1·25	7·98	3·71	3·0	0·022	−56·4	−37·6	+45·6
90		210	2·09	7·98	7·65	3·7	0·017	−70·4	−53·7	—
104	**P XIV. "O.M."**	78	1·43	7·98	3·2	2·2	0·029	−72	−58·4	+36·2
105		124	1·47	7·98	5·17	3·5	0·028	−73·6	−66·1	—
106		155	1·56	7·98	5·72	3·7	0·024	−70·4	−57·0	+23·8
107	**P XIV**	79	1·30	7·98	4·59	3·5	0·045	−48	−46·8	+45·5
108		124	1·20	7·98	5·31	4·4	0·036	−51·2	−36·1	—
109		155	1·22	7·98	5·44	4·5	0·029	−72	−60·2	+19·6
101	**P II**	99	0·7	7·98	4·09	5·8	0·059	−55·2	−84·3	+22·5
98		134	1·4	7·98	7·63	5·4	0·041	−72	−100	−0·4
100		222	0·88	7·98	7·42	8·4	0·038	−63·2	−75·1	—
22	**H 12**	35	0·23	7·2	0·18	0·8	0·022	+ 9·7	+ 4·0	
20		129	1·68	7·2	5·62	3·3	0·026	−35·9	−71·1	
21		198	1·59	7·2	6·76	4·2	0·021	+16·3	− 6·7	

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* In experiment 126, air was repeatedly passed over the surface of the culture during incubation.

TABLE XVI—*continued.*

No. 1.	Virus. 2.	Age. 3.	Weight. 4.	Glycerol in Medium. 5.	Glycerol Used. 6.	Glycerol per Gram of Culture. 7.	Glycerol per Gram per Day. 8.	Change in Acidity. 9.	Nett Change in Acidity. 10.	Residual Change in Acidity. 11.
25	H 27	35	0·42	7·2	0·13	0·3	0·009	−25·1	−35·5	
24		129	1·66	7·2	5·96	3·6	0·028	+ 3·7	−31·9	
66	H 49	188	1·59	7·55	7·41	4·7	0·025	−40·2	−31·5	
53	H 49	105	2·2	8·3	5·2	2·4	0·0225	−64·6	−94·6	
52		240	1·47	8·3	7·99	5·4	0·0226	−29·4	−30·4	
44	H 74	114	2·4	7·55	5·3	2·2	0·019	−57·8	−103·7	
43		200	1·1	7·55	5·97	5·4	0·027	+79·8	+58·8	
42		269	1·57	7·55	7	4·5	0·016	− 3·4	+42·6	
117	H 74	26	0·35	7·38	0·01	0·03	0·001	−18·4	−33·5	+12·3
116		111	1·55	7·38	5·96	3·8	0·035	−63·2	−85·3	− 8·7
*118	H 74	80	0·68	7·38	1·3	1·9	0·024	−21·4	−22·4	+64·4
56	H 75	240	1·29	8·3	8·13	6·3	0·026	−50·2	−47·8	
87	H 2	114	1·81	12·4	8·21	4·5	0·040	+12·4	−17·5	
88		166	2·18	12·4	9·32	4·3	0·026	+ 7	− 2·2	
89		232	2·2	12·4	11·64	5·3	0·023	+12·6	+26·1	
85	H 19	93	1·18	12·4	4·83	4·1	0·044	+23·8	+43·1	
84		178	2·07	12·4	8·56	4·1	0·023	−13·4	+ 5·2	
83		254	1·44	12·4	10·37	7·2	0·028	− 1·8	+35·7	
64	H 31	142	2	20·36	7·06	3·5	0·025	−35·5	−44·8	+ 8·4
*76	H 31	145	1·57	20·36	8·48	5·4	0·037	− 4·7	−10·5	+30·8
119	H 90	27	0·64	7·38	0·35	0·55	0·02	−43·2	−48·0	+15·3
120		62	1·04	7·38	2·95	2·8	0·046	−39·2	−64·1	+10

* In experiments 76, 118, air was repeatedly passed over the surface of the culture during incubation.

Relation of glycerol consumed to carbon dioxide produced.—Three experiments were carried out in which moist sterile air, freed from carbon dioxide, was repeatedly passed over the culture during incubation and the carbon dioxide which was produced absorbed in caustic potash and estimated.

The following are the results obtained.

TABLE XVII.

Number.	Strain.	Age of Culture.	Weight of Bacillus.	Original Glycerol in Medium.	Glycerol Used.	CO_2 formed.	Glycerol Equivalent to CO_2.	Glycerol not Accounted for.	Percentage of Glycerol used Oxidised.
76	H 31	145	1·57	20·4	8·5	6·6	4·6	3·9	54·1
118	H 74	80	0·68	7·38	1·3	0·68	0·47	0·83	36·1
126	B X	130	0·68	7·98	1·11	0·73	0·49	0·62	44·1

In every case the total carbon dioxide is considerably less than that which would be produced by the complete oxidation of the glycerol used. Even if the whole production of carbon dioxide be ascribed to the oxidation of glycerol, an assumption for which there is no adequate ground, the result attained is that from 36 to 54 per cent. of the carbon of the glycerol used is converted into carbon dioxide. In view of the fact that there are other sources of carbon abundantly present, these numbers must be regarded as showing the maximum amounts of the glycerol used which can have been completely oxidised. No attempt has yet been made to ascertain what becomes of the glycerol which disappears without being oxidised. In experiment 76, the amount of glycerol still to be accounted for is 3·9 grammes, containing 1·52 grammes of carbon, and this is more than can have been assimilated in the production of 1·57 grammes of bacillus. In the other two experiments the residual amounts of glycerol (0·83 g. and 0·62 g.) are so small that they might possibly be accounted for in this way.

SUMMARY AND CONCLUSIONS.

1. No definite physiological difference has been detected between tubercle bacilli of different origins.
2. Such differences as exist between the amounts of action exerted on glycerol beef broth by different cultures are probably to be attributed to differences between the weights of organism formed, the times of incubation and individual characteristics of the strains.
3. When B. Tuberculosus is cultivated on glycerol broth, the proteins of the broth undergo hydrolysis to a considerable extent.
4. Glycerol is partially removed by oxidation. There is no evidence that consumption of glycerol is directly related either to weight of culture obtained or to change in acidity.
5. The initial fall in the acidity of the medium is largely due to the removal of the acids soluble in ether (lactic acid, etc.).
6. Further important factors in producing change in acidity are the production and removal of ammonia (free and saline) and the digestion of the proteins.

PART II.

THE RELATIVE AMOUNTS OF ASH AND PHOSPHORIC OXIDE IN TUBERCLE BACILLI OF DIFFERENT ORIGINS.

This investigation was undertaken with the object of ascertaining whether tubercle bacilli of different origins and characteristics showed any definite and constant differences between the proportions of phosphoric oxide and mineral ash respectively which they contained. De Schweinitz and Dorset (1) found as the result of a similar comparison, in which one culture of each of six viruses was analysed, that bacilli of human origin (both attenuated and virulent) yielded an ash containing a larger proportion of phosphoric oxide than those derived from bovines, swine, horses, and fowls. These results are quoted in detail on p. 33, and it was to ascertain whether the organisms obtained by the Royal Commissioners exhibited similar differences that the following analyses were made.

1. *Preparation of the medium.*—The medium consisted of ordinary glycerol peptone veal broth to which 0·5 gram of potassium dihydrogen phosphate was added per 100 cc. in place of 0·5 gram of common salt, in accordance with the recommendation of De Schweinitz and Dorset (1). The broth was prepared in the manner previously described, but it was found that the addition of the phosphate caused a deposit to be formed when the clear liquid was brought to a reaction + 15 and sterilised. This precipitate was removed by filtration but a fresh deposit was formed during sterilisation and it was found necessary to repeat these processes of filtration and sterilisation about six times before a medium was obtained which yielded a clear liquid after sterilisation and cooling. The acidity was then found to be + 48, and although this was somewhat high, the medium was employed without further treatment as it was found that the addition of alkali caused renewed precipitation.

The liquid as actually employed was found to contain, according to two analyses (*a*) 0·3666, (*b*) 0·3716 grams of phosphoric oxide per 100 cc. It may be noted for comparison that four samples of non-phosphated broths, which were examined, contained the following amounts of phosphoric oxide per 100 cc.

Medium 9	0·1198 grams.
Medium 11	0·0722 grams.
Medium 12	0·1138 grams.

2. *Method of cultivation.*—The broth was placed in quantities of 125 cc. in Erlenmeyer flasks of 900 cc. capacity, so that it formed a layer about 1 cm. deep. It was inoculated in the usual way and the mouths of the flasks were plugged with cotton wool, over which was placed a sheet of sterilized tin-foil to prevent evaporation. Four of the strains were grown at Stansted under the care of Drs. S. and H. E. Griffith, to whom my thanks are due for their kindness in undertaking this part of the work, whilst the remaining five strains were grown at the Lister Institute. Eight flasks of each of the virulent strains and six of each of the non-virulent strains were inoculated. When no growth occurred supplementary flasks were inoculated at a later date, but these did not in all cases produce growths. No attempt was made to obtain growths of precisely equal age, as the uncertainty of the times of commencement and cessation of growth was too great to permit of this being done with any accuracy.

3. *Details of the cultures employed*:—

Cultures Employed.		Inoculated.	Removed.
1. Virus B II. Heifer 66. 31st Generation. 12/12/07	...	11/3/08	8/5/08
2. Virus B X. G.-P. 1070. 45th Generation.	3 Flasks	24/2/08	30/4/08
	5 Flasks	1/4/08	30/4/08
3. Virus P LVIII. 43th Generation.	4 Flasks	29/2/08	30/4/08
	4 Flasks	1/4/08	30/4/08
4. Virus H 88. "E.L." 17th Generation	... ...	22/2/08	30/4/08

Cultures Employed.	Inoculated.	Removed.
5. Virus H 19. "S.W." Goat 11. 24th Generation ...	24/2/08	30/4/08
6. Virus H 68. "R.B." Calf 1247. Prepectoral gland 14/3/08—27/3/08. 2 Flasks	7/4/08	2/7/08
3 Flasks	11/5/08	2/7/08
7. Virus H 98. "B.R." G.-P. 2878. Pyloric gland 14/3/08—27/3/08. 2 Flasks	20/4/08	2/7/08
2 Flasks	11/5/08	2/7/08
8. Virus A VIII. Fowl B. Zoo XI. 8th Generation. March 4. 4 Flasks	20/4/08	6/7/08
4 Flasks	11/5/08	6/7/08
9. Virus A X. 11th Generation. March 4. 4 Flasks	20/4/08	6/7/08
2 Flasks	11/5/08	6/7/08

4. *Treatment of the cultures preparatory to analysis.*—The strains of human and bovine origin were filtered through Schleicher and Schull's No. 595 paper in the cold, and were then washed with cold water until free from broth. They were next placed in the steam steriliser for half an hour, and were finally washed on the filter paper with boiling water until the filtrate gave no reaction for chlorides with silver nitrate or for phosphate with ammonium molybdate.

The growth was then removed, while still moist, from the filter paper, placed in a watch glass and exposed over sulphuric acid in a vacuum until it was dry enough to be powdered. The mass was then ground in an agate mortar, placed in a weighed weighing bottle, and again exposed over sulphuric acid in a vacuum until no further loss of weight occurred, this final exposure lasting several months.

The two strains of avian origin were collected and freed from broth by being centrifugalised, and the deposit mixed with cold water and again centrifugalised. They were then washed into a flask, sterilised, and treated in exactly the same way as the others.

5. *Analysis of the dried bacilli.*—Owing to the comparatively small weight of the organism obtained in each case, it was necessary to limit the analysis to the estimation of the ash and phosphoric oxide. The dry material was weighed out into a weighed platinum basin and incinerated. It was found impossible to remove the carbon, which formed a hard, coke-like mass, at any lower temperature than a bright red heat, and the heating was continued in all cases until constancy of weight was obtained. The ash was then treated on the water-bath in the platinum basin with 5 cc. of concentrated sulphuric acid and two successive quantities of 25 cc. of strong nitric acid to render solution complete and at the same time to convert any metaphosphate into orthophosphate. Water was then added, the liquid filtered, 15 cc. of magnesium citrate mixture added, followed by excess of ammonia, and the whole stirred and allowed to stand over night. It was then filtered, and the precipitate washed with dilute ammonia, dried, and weighed after ignition as magnesium pyrophosphate ($Mg_2P_2O_7$). This method of igniting, and estimating the phosphate in the ash, gave results identical with those obtained by treating the bacilli directly with sulphuric and nitric acids (Neumann's method) and estimating the phosphoric oxide in the resulting solution by means of magnesium citrate. This is shown by analyses carried out by both methods of the growth obtained from Virus B X and from another growth A, not included in the present series.

—	Per cent. of P_2O_5 in Bacillus.	
	B X	A
(1) By analysis of ash	1·33	2·26
(2) By direct analysis	1·36	2·23

6. *Results.*—The results obtained are tabulated below.

TABLE I.

Strain.	Amount taken for Analysis.	% of Ash.	% of P_2O_5 in Ash.	% of P_2O_5 in Bacillus.
BOVINE, OF BOVINE OR PORCINE ORIGIN.				
B II	(*a*) 0·9469	3·44	68·9	2·37
	(*b*) 0·8445	3·27	67·3	2·20
B X	(*a*) 0·9819	2·40	55·4	1·33
	(*b*) 0·8207	By direct analysis.		1·36
P LVIII	1·0524	2·24	54·8	1·23
BOVINE, OF HUMAN ORIGIN.				
H 88. "E.L." ...	1·3871	2·37	65·3	1·55
H 19. "S.W." ...	1·0429	2·92	65·0	1·90
HUMAN.				
H 68. "R.B." ...	1·5736	3·29	64·1	2·10
H 98. "B.R." ...	1·0876	3·02	66·7	2·01
AVIAN.				
A VIII	1·2882	3·47	63·6	2·21
A X	0·6304	3·17	65·0	2·06

In considering these numbers it must be borne in mind that, owing to the conditions of the determination, the limits of accuracy are from 0·1 to 0·2 in the percentages of ash and phosphoric oxide in the bacillus, and about twenty times this, 2 to 4, in the percentages of phosphoric oxide in the ash.

1. *Percentage of ash.*—The percentages of ash form a gradual series extending from 2·24 to 3·47. Of the bovine viruses, two (P LVIII and B X) are low, whilst one (B II) is high. Between the two bovine viruses of human origin there is a very definite difference of 0·55 per cent. The two human viruses have about equal percentages of ash, and the same is true approximately of the two avian viruses.

When the strains are arranged in the order of magnitude of the percentage of ash it is found that two of the bovine viruses and one of the bovine viruses of human origin form a group having the lowest percentage, and these are followed, after a marked gap in the series, by a second group comprising the other bovine virus of human origin, the two human viruses and the two avian viruses, with which is associated the remaining bovine virus, in order of increasing percentage of ash, as shown in the following table :—

TABLE II.

Viruses arranged in order of increasing percentages of ash.

Virus.	Percentage of Ash.	Virus.	Percentage of Ash.
P LVIII	2·24	H 98. "B.R."	3·02
H 88. "E.L."	2·37	A X	3·17
B X	2·40	H 68. "R.B." ...	3·29
		B II	3·35
H 19. "S.W."	2·92	A VIII	3·47

II. *Percentage of phosphoric oxide in the bacillus.*—Very similar relations are shown by these numbers. They form a series from 1·23 to 2·28 per cent. and may be roughly divided into the same two groups as the percentages of ash.

Table III.

Viruses arranged in order of increasing percentages of phosphoric oxide in the bacilli.

Virus.	Percentage of Phosphoric Oxide in Bacilli.	Virus.	Percentage of Phosphoric Oxide in Bacilli.
P LVIII	1·23	A X	2·06
B X	1·34	H 68. "R.B." ...	2·10
H 88. "E.L."	1·55	A VIII	2·21
H 19. "S.W."	1·90	B II	2·28
H 98. "B.R."	2·01		

III. *Percentage of phosphoric oxide in the ash.*—These numbers also fall into two chief groups :—

(*a*) Comprising two bovine viruses for which the percentage is about 55 ;

(*b*) Comprising the remaining bovine virus, which has the high percentage 68, and all the others, the percentages ranging from 64 to 67.

Table IV.

Viruses arranged in order of increasing percentages of phosphoric oxide in the ash.

Virus.	Percentage of Phosphoric Oxide in the Ash.	Virus.	Percentage of Phosphoric Oxide in the Ash.
P LVIII	54·8	H 19. "S.W." ...	65
B X	55·4	H 88. "E.L." ...	65·3
A VIII	63·6	H 98. "B.R." ...	66·7
H 68. "R.B."	64·1	B II	68·1
A X	65		

It will be seen that no definite distinction between viruses of different origin can be established on these numbers. Thus whilst two of the bovine viruses and one of the bovine viruses of human origin appear to agree in percentage of ash, the percentage of phosphoric oxide in the ash is considerably higher for the virus of human origin. On the other hand the second bovine virus of human origin must be classed along with the human, the avian and the third bovine virus both in percentage of ash and percentage of phosphoric oxide in the ash.

7. *Comparison with previous results.*—The only series of comparative estimations of ash and phosphoric oxide in tubercle bacilli of different origins is that due to de Schweinitz and Dorset (1), which served as the model of the foregoing experiments. The results of these investigators are in general qualitative agreement with those stated above.

Bacilli of six different origins were analysed with the following results :—

Table V.

De Schweinitz and Dorset's results.

Origin.	Moisture.	Ash.	Phosphoric Oxide in Dry Bacilli.	Phosphoric Oxide in Ash.
Bovine	2·43	2·66	1·56	58·29
Swine	2·16	2·34	1·30	55·74
Horse	2·34	3·59	2·04	55·54
Avian	2·40	3·95	2·20	55·81
Human (attenuated)	2·62	2·37	1·75	73·93
Human (virulent)	3·80	3·93	2·44	62·18

The relations between the human attenuated and human virulent of these authors and the slightly virulent and virulent human viruses of the Royal Commission are not quite clear, and this renders any strict comparison between the two sets of results impossible. It is however to be noted that the virus with the highest percentage of phosphoric oxide in the ash has almost the lowest percentage of ash and a comparatively low percentage of phosphoric oxide in the bacillus itself. The avian bacillus examined by de Schweinitz and Dorset yielded an ash with a considerably lower percentage of phosphoric oxide than those examined by me, whilst the ash of their bovine bacillus agrees approximately in composition with those of two of the bovine viruses examined by me, but differs from that of the third.

The fact that the composition of the ash derived from viruses of bovine origin may differ so widely indicates the necessity for the examination of a large number of viruses before any conclusion can be safely drawn on this point.

It must also be remembered that many other factors may be concerned in the question, particularly the exact composition of the medium, the duration of growth, and the past history of the culture. The only one of these which has been investigated is the first, with the result that the composition of the bacillus and of its ash were found to vary very widely with the nature of the medium [de Schweinitz and Dorset (2), Levene (1)].

Under these circumstances and having regard to the differences between the nature of the results obtained by de Schweinitz and Dorset and myself, it appears to be impossible definitely to characterise tubercle bacilli of different origins by the amount or composition of the ash which they yield or by the amount of phosphoric oxide contained in the bacillus.

Summary.

It appears probable from a consideration of all the material available that the percentage of ash and of phosphoric oxide in the tubercle bacillus, as well as the composition of the ash depend on so large a number of factors that until a careful investigation of all these has been made, it is impossible to utilise these numbers for the characterisation of viruses of different origins.

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PART II. APPENDIX.

VOLUME VI.

Report on the Results of a Chemical Investigation.

DR. ARTHUR HARDEN, F.R.S.

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(27676—i.) Wt. 13670/04—5. 1250. 6/15. D & S.

PREFACE.

Six volumes of Appendix to the Final Report of the Royal Commission on Tuberculosis have already been published.

These volumes which record in detail the researches on which the Commission based their findings were issued as quickly as was possible after the presentation of the Final Report. A small and final volume of appendix has been delayed in circumstances as follows :—

The particular investigations graphically illustrated in this volume have been almost entirely of the kind known as passage experiments. Many of them covered long periods of time, and extension of some of them might profitably have been continued after the indispensable practical work of the Commission at Stansted was brought to a close. Though the work of the Commission was then regarded as complete, so far as it related to the actual terms of reference, it was realised that certain of the investigations had afforded indication of problems which, though outside the scope of the Commission's reference, deserved to form the subject of further research in the interests of some wider aspects of the tuberculosis question.

It was hoped that arrangements would be made by which the work commenced by the Commission would be continued without much delay, and that the material and equipment collected and organised in the course of its labours would be utilized by competent investigators. Hence the issue of this volume was purposely delayed pending continuance under due authority of the work at Blythwood Farm, in order that, if possible, some statement might be made in it as to the class of experiments which would thus have been further extended.

Unfortunately no such arrangements for the continuation of the work of the Commission materialised, and though in several respects lupus research has been further extended it is not proper to refer here to the additional results thus obtained as the work was not carried out at Government expense.

The delay that has thus arisen in the issue of this last volume of the Appendix to the Final Report of the Tuberculosis Commission, for which delay the Secretary would have himself regarded as wholly responsible, is not important from the scientific point of view. No facts are here graphically set out that have not, as facts, been already recorded in other parts of Appendix or in the Final Report of the Commission.

The contents of the volume itself require little explanation. The method of setting out the results of passage experiments as charts was devised by Dr. Cobbett, and was adopted by the Commission in the Appendix to its second Interim Report. This method was subsequently extended so as to depict the distribution and extent of the lesions found in experimental animals after death. The lesions observed in the animal were delineated in each instance on the chart immediately after the post-mortem examination was made.

Completed charts of all the more important experiments having been reproduced are here collected together.

These charts proved of great value during the course of the experimental work, and subsequently, when the results were being collated, in at once affording a clearer mental picture of the results of a series of experiments than was possible from a perusal of the post-mortem notes.

It is anticipated that they will also be of great assistance in helping those interested to a better understanding of the many and complicated passage experiments of the Commission.

The diagrams illustrating the experiments (pages 1 to 29 in this volume) recorded in the Appendices to the Second Interim Report were drawn by Dr. Cobbett. Those illustrating the experiments detailed in the Appendices of the Final Report were drawn, under supervision, by Mr. C. F. Fox, to whom was entrusted also the correction of the lithographer's proofs and the collection from the various Appendices of the details used in the descriptions of the charts.

In the diagrams usually only those organs and glands are outlined in which lesions were observed. When the organ or gland was found enlarged the outline in the diagram was made proportionately larger.

Solid organs (liver, spleen, and kidneys) are shaded grey, and grey shading is used in some of the lung outlines to represent areas of hepatisation.

The method of representing the extent and the severity of the tuberculosis produced is by means of red contour lines and spots.

Contour lines are used for tissues, such as the local lesion, the adjacent glands and the thoracic glands or their cortices, which showed uniform tuberculous infiltration.

Lymphatic glands, which, like the portal, renal, or prepectoral glands, were too small as figured in the diagram to contain contour lines are, when uniformly tuberculous, depicted entirely in red.

The red spots indicate tubercles and nodules. The size of the spots bears broad relation to the size of the actual lesions. It has not, however, been possible to make reproducible distinction between tubercles below a certain size, i.e. about that of a millet seed, all of which are represented by fine dots. For nodules larger than a millet seed the dots are heavier in proportion to the size of the nodule.

When whole lobules in the lungs were tuberculous these are represented by angular red spots of various sizes.

Red circles on the lungs and liver or around their margins and on the omentum represent "perlsucht" growths.

The density of the dots within the outlines of the different organs is roughly a measure of the severity of the tuberculous process.

There is not, however, exact numerical relationship between the number of dots in different diagrams and the number of actual tubercles. When there were only one or two tubercles in an organ each is represented in the diagram by a dot, but when the tubercles were moderately or extremely numerous it was obviously impossible to depict every tubercle. Slight disease, therefore, is from the numerical standpoint unduly emphasised in the diagrams.

E. J. S.

NOTE.—Abbreviations used in the graphic charts :—

G.P.—Guineapig.

Subcut.—Subcutaneous inoculation.

Intrap.—Intraperitoneal inoculation.

Intramamm.—Intramammary inoculation.

T.B.—Tubercle bacilli.

TABLE OF CONTENTS.

1.—Twenty-five Charts illustrating the Investigation of Twenty-four Viruses of Human Origin presenting definite features produced by either the Bovine or the Human Tubercle Bacillus (Group I. or Group II.) recorded in the 2nd Interim Report.

For details, *see* Appendix to 2nd Interim Report, Vol. II. (Dr. Cobbett).

List of Viruses Illustrated :—

		Reference No. of Chart.			Reference No. of Chart.
Group I. : (*Bovine tubercle bacilli*).	H. 2. " Sp. A."	1	**Group II.** : (*Human tubercle bacilli*).	H. 8. " S.C."	12
	H. 7. " C.M."	2		H. 9. " C.T."	13
	H. 10. " B.S." (two charts)	3 & 3A		H. 11. " E.D."	14
	H. 14. " F.S."	4		H. 12. " H.N."	15
	H. 19. " S.W."	5		H. 15. " I.W."	16
	H. 20. " F.L."	6		H. 18. " T.T."	17
	H. 28. " C.L."	7		H. 22. " F.W."	18
	H. 29. " M.F."	8		H. 23. " J.P."	19
	H. 31. " L.F."	9		H. 25. " A.T."	20
	H. 32. " Y.W."	10		H. 26. " K.M."	21
	H. 59. " L.B."	11		H. 27. " B.D."	22
				H. 30. " E.M."	23
				H. 36. " M.D."	24

2.—Ten Charts illustrating Five Viruses provisionally classed in Group III. in the 2nd Interim Report;

One Chart illustrating the further Investigation of Two of these Viruses; and

One Chart illustrating Two Viruses subsequently investigated which presented similar anomalies.

For details, *see* Appendix to 2nd Interim Report, Vol. II. (Dr. Cobbett), and Appendix to Final Report, Vol. I. (Dr. A. S. Griffith).

List of Viruses Illustrated :—

	Reference No. of Chart.
H. 13. " A.D." (two charts)	25 and 25A.
H. 16. " J.H." (two charts)	26 and 26A.
H. 17. " Sp. B." (three charts)	27, 27A, and 27B.
H. 21. " G.B." (two charts)	28 and 28A.
H. 49. " T.C." (one chart)	29
H. 13. " A.D." and H. 49. " T.C." (further investigation) (one chart)...	30
H. 60. " W.B." ... H. 90. "I.P." ... (one chart)	31

3.—Two Charts illustrating Fourteen Viruses, investigated after the issue of the Second Interim Report, showing typical features of Group I. or Group II.

For details, *see* Final Report, Appendix Vol. I. (Dr. A. S. Griffith).

Contents of the Charts :—

One Chart :— (No. 32)	" Inoculations in calves with the six virulent human viruses" (Viruses H. 69, H. 77, H. 88, H. 89, H. 127, (*a*) and (*c*), H. 128 (*a*) and (*b*).)
One Chart :— (No. 33)	" Inoculations in calves with tissue emulsions and cultures from slightly virulent human viruses." (Viruses H. 67, H. 74, H. 75, H. 78, H. 79, H. 80, H. 81, H. 87, H. 104.)

4.—Seven Charts illustrating the Investigations by Dr. A. S. Grffith of Nineteen Lupus Viruses, together with one Lupus Virus (H. 53)* investigated by Dr. Cobbett, and reinvestigated by Dr. Griffith.

For details, *see* Final Report, Appendix Vol. II. (Dr. A. S. Griffith) and 2nd Interim Report, Appendix Vol. II. (Dr. Cobbett).

CONTENTS OF THE CHARTS :—

One Chart (No. 34) :—“ Inoculations in calves with four of the lupus cultures which grow like bovine tubercle bacilli.” (Viruses H. 110, H. 100, H. 111, H. 91.)

One Chart (No. 35) :—“ Inoculations in calves and passage experiments with two of the lupus cultures which grow like bovine tubercle bacilli.” (Viruses H. 105 and H. 107.)

One Chart (No. 36) :—“ Test inoculations in calves and passage experiments with two of the lupus cultures which grow like bovine tubercle bacilli.” (Viruses H. 85 and H. 108.)

One Chart (No. 37), showing the original inoculation and passage experiments with the lupus virus H. 53. “ D.H.” (*a*), carried out by Dr. Cobbett and reported in the 2nd Interim Report.

One Chart (No. 37A) :—“ Test inoculations in calves and passage experiments with the lupus virus H 53. “ D.H.” (*a*) and (*b*), the cultures from which grow like bovine tubercle bacilli.”

One Chart (No. 38) :—“ Culture inoculations in calves of the eleven easy growing lupus viruses.” (Viruses H. 102, H. 103, H. 106, H. 84, H. 101, H. 114, H. 71, H. 112, H. 109, H. 92, H. 99.)

One Chart (No. 39) :—“ Pigs and Goats inoculated with cultures from three lupus viruses ” and “ Pigs and Goats inoculated with Group II. Cultures (included for comparison with above).”

5.—Five Charts illustrating the investigation of Four Equine Viruses.

For details, *see* Final Report, Appendix Vol. IV., Dr. F. Griffith.

CONTENTS OF THE CHARTS :—

Two Charts (Nos. 40 & 40A) :—Inoculation Experiments on Calves with Culture of Virus E. II.

Two Charts (Nos. 41 & 41A) :—Inoculation Experiments on Calves with Culture of Virus E. IV.

One Chart (No. 42) :—
Inoculation Experiments on Calves with Cultures of Viruses E. I. and E. III.
Inoculation Experiments on Pigs with Viruses E. I., E. II., E. III., and E. IV.

---

* Provisionally classed in the 2nd Interim Report in Group III.

1.

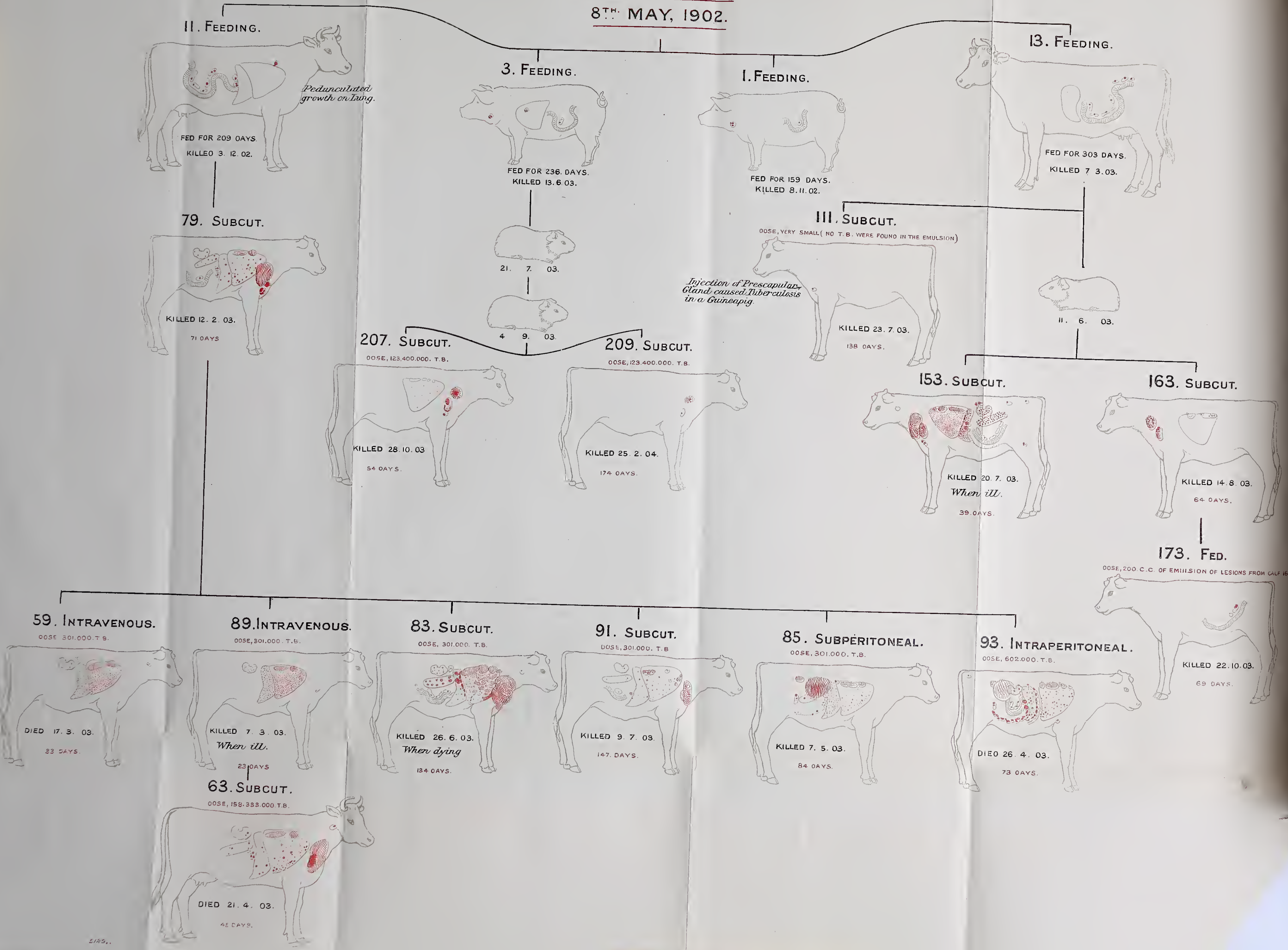
25 Charts illustrating the investigation of 24 viruses of human origin presenting definite features produced by either the bovine or the human tubercle bacillus, recorded in the 2nd Interim Report.

Pages 5 to 29

VIRUS H. 2. "SP. A."

HUMAN SPUTUM.

8TH. MAY, 1902.



[illegible] Ld. Lith. London

CHART 1.

VIRUS H. 2. "Sp. A." Human Sputum. May 8, 1902.

The original material of this virus was the mixed sputum of many individuals, which was subsequently found to contain **bovine as well as human tubercle bacilli.**

Nature of Disease : *Pulmonary Tuberculosis.*

Material used (*clinical*) : Mixed Sputum from many patients.

Explanation of Chart.—Experiments with this virus were made for the purpose of determining virulence. Bovine animals and pigs were fed with the sputum, and passage experiments were carried out with the tubercle bacilli contained in tuberculous material derived from these animals. *Emulsion* of tuberculous lesions was alone employed for passages from animal to animal.

It was shown that virulent (bovine) tubercle bacilli were present in the lesions of at least one of the fed animals (Heifer 11).

As a result of the investigation of viruses which showed similar anomalies, the Commission concluded that the results obtained are to be accounted for by the presence in the human sputum as used for feeding of a small number of bovine tubercle bacilli.

Experiments on calves with *cultures* obtained at different stages of the passage experiments depicted in Chart 1, were also made. (*See* reference in italics.)

References.—2nd Interim Report : Page 18, para. 34 ; page 25, para. 43 ; page 33, para. 58 ; page 34, paras. 60–65.

Appendix to 2nd Interim Report : Vol. II., pages 4 and 47–92 ; *Chart of Culture Inoculations*, page 49.

Final Report, page 14.

Appendix to Final Report, Vol. III. : "Investigation of Certain Human Viruses of Irregular Type."

VIRUS H. 7. "C.M."

MESENTERIC GLANDS.

NOVEMBER 11TH 1902.

5183.

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CHART 2.

VIRUS H. 7. "C.M." Mesenteric Glands. November 11, 1902.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature case : *Primary Abdominal Tuberculosis.*

Age of patient : 3 years.

Material used (*post-mortem*) : Mesenteric glands only.

Explanation of Chart.—The chart illustrates (1) the inoculation experiments on bovine animals with *emulsions* of the mesenteric glands, undertaken to determine the original virulence of the virus ; and (2) experiments to test the effect of passage from animal to animal. (In these experiments *emulsions* of tuberculous tissues were alone employed.)

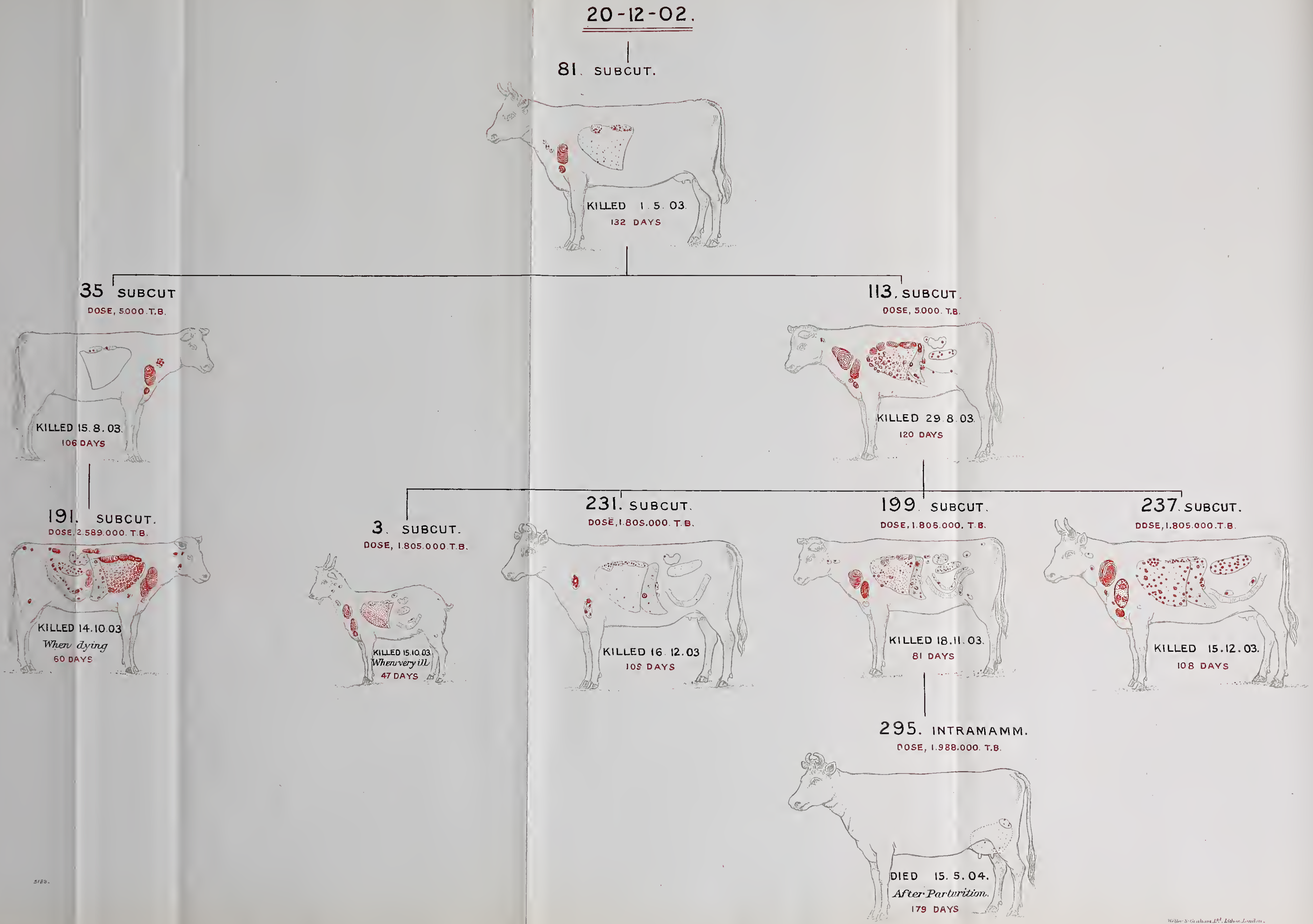
Experiments on calves with *cultures* of this virus, derived indirectly from the original material, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 14, paras. 21 to 27 ; page 25, paras. 43 and 44 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 10 and 119 to 158 ; *Table of Culture Inoculations*, page 120

Final Report, pages 9 to 13.

MESENTERIC GLANDS. VIRUS H. 10. "B. S."



3185.

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CHART 3.

VIRUS H. 10. "B.S." Mesenteric Glands. December 20, 1902.

A virus obtained from a case of tuberculosis in man, subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature of case : *Primary Abdominal Tuberculosis.*

Age of Patient : 1 year 9 months.

Material used (*post-mortem*) : Mesenteric Glands only.

Explanation of Chart.—The chart illustrates (1) the inoculation experiment on a heifer (81) with *emulsion* of the mesenteric glands, made to determine the original virulence of the virus ; and (2) further experiments to test its virulence for the calf and goat and to determine the effect of passage from animal to animal. (In these experiments *emulsions* of tuberculous tissues were alone employed.)

References.—2nd Interim Report : Page 14, paras. 21 to 37 ; page 25, paras. 43 and 44 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 10 and 201 to 244.

Final Report, pages 9 to 13.

See also Chart 3A for inoculation experiments with *cultures* of this virus.

VIRUS H.10. "B.S."

CULTURE INOCULATIONS.

STRAIN DERIVED FROM CALF 113, THROUGH G.P. 757.

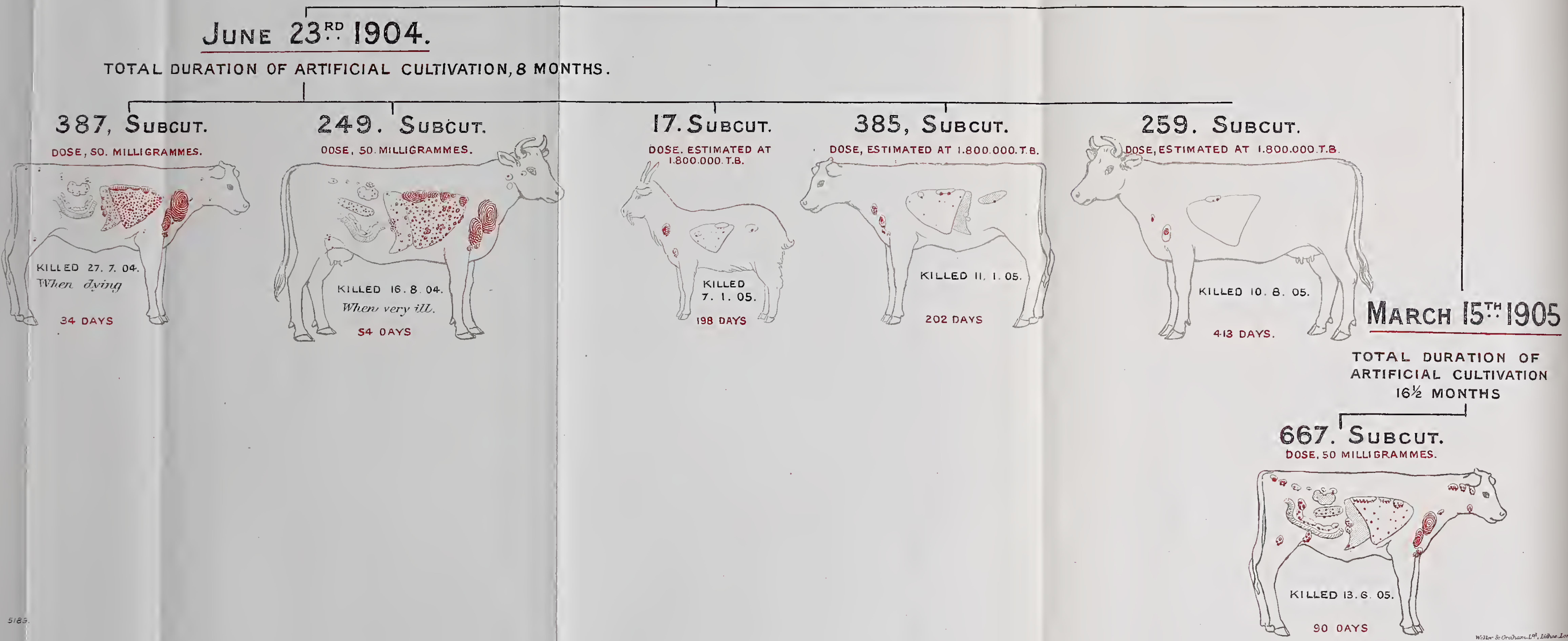

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CHART 3A.

VIRUS H. 10. "B.S." Culture Inoculations; strain derived from Calf 113 through Guinea-pig 757. (*See* Chart 3.)

Explanation of Chart.—The chart illustrates the inoculation experiments on bovine animals and a goat undertaken to test the relative virulence of *cultures* and tissue *emulsions* of the same virus. (For tissue emulsion inoculations *see* Chart 3.)

References.—2nd Interim Report : Pages 12 and 13, para. 20.

Appendix to 2nd Interim Report, Vol. II. : Page 203 *et seq.*

MESENTERIC GLANDS. VIRUS. H. 14. "F. S."

JUNE 8TH 1903.

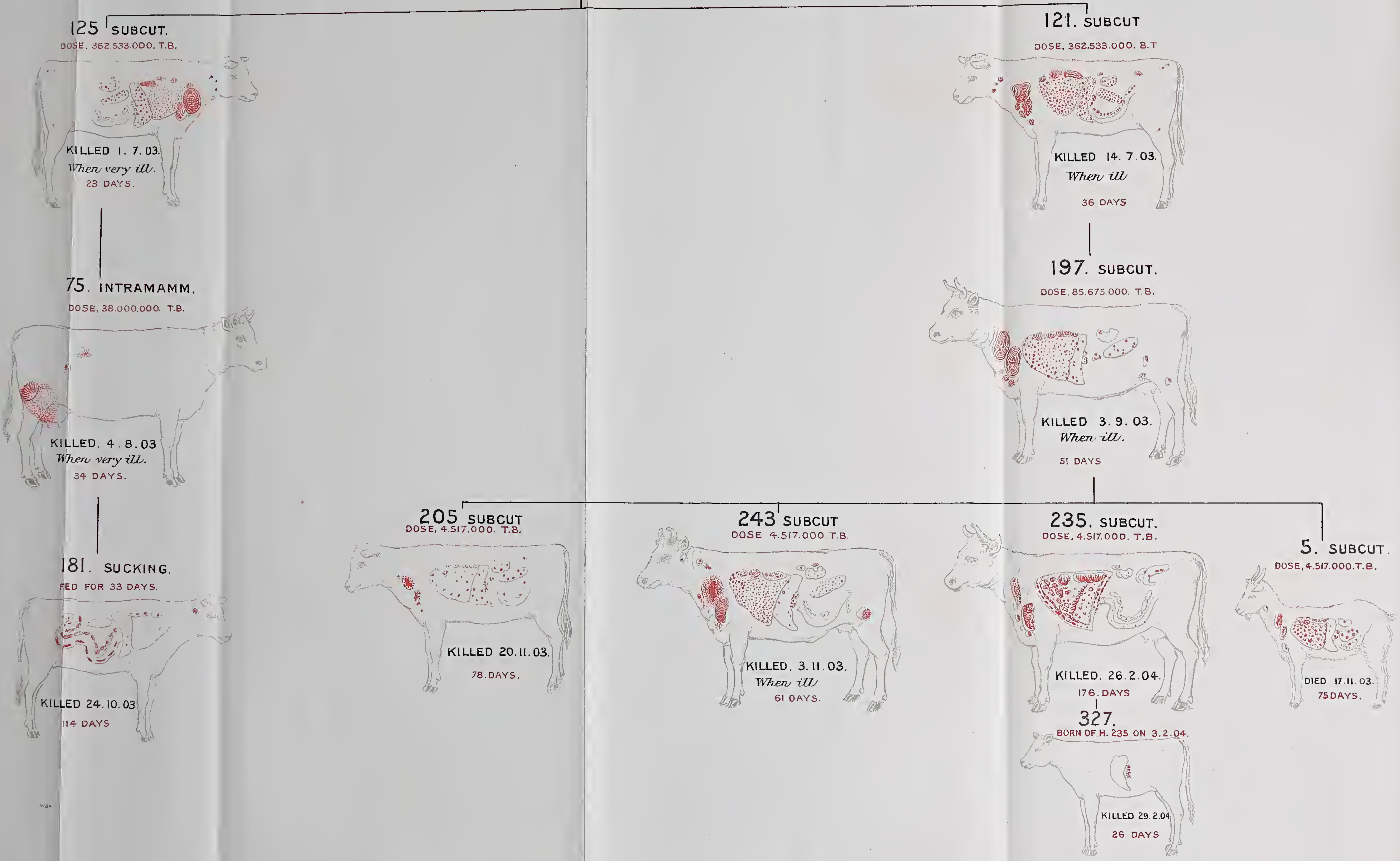


CHART 4.

VIRUS H. 14. "F.S." Mesenteric Glands. June 8, 1903.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature of case : *Primary Abdominal Tuberculosis.*

Age of patient : 4½ years.

Material used (*post-mortem*) : Mesenteric Glands only.

Explanation of Chart.—The Chart illustrates (1) the inoculation experiments on bovine animals with *emulsion* of the mesenteric glands, undertaken to determine the original virulence of the virus ; and (2) experiments to test the effect of passage from animal to animal, the effect of intramammary inoculation and ingestion, the virulence for the goat, &c. (In these experiments *emulsions* of tuberculous tissues were alone employed.)

Experiments on calves with a *culture* of the virus, derived from the original material, through the guinea-pig, were also carried out. (*See* reference in italics.)

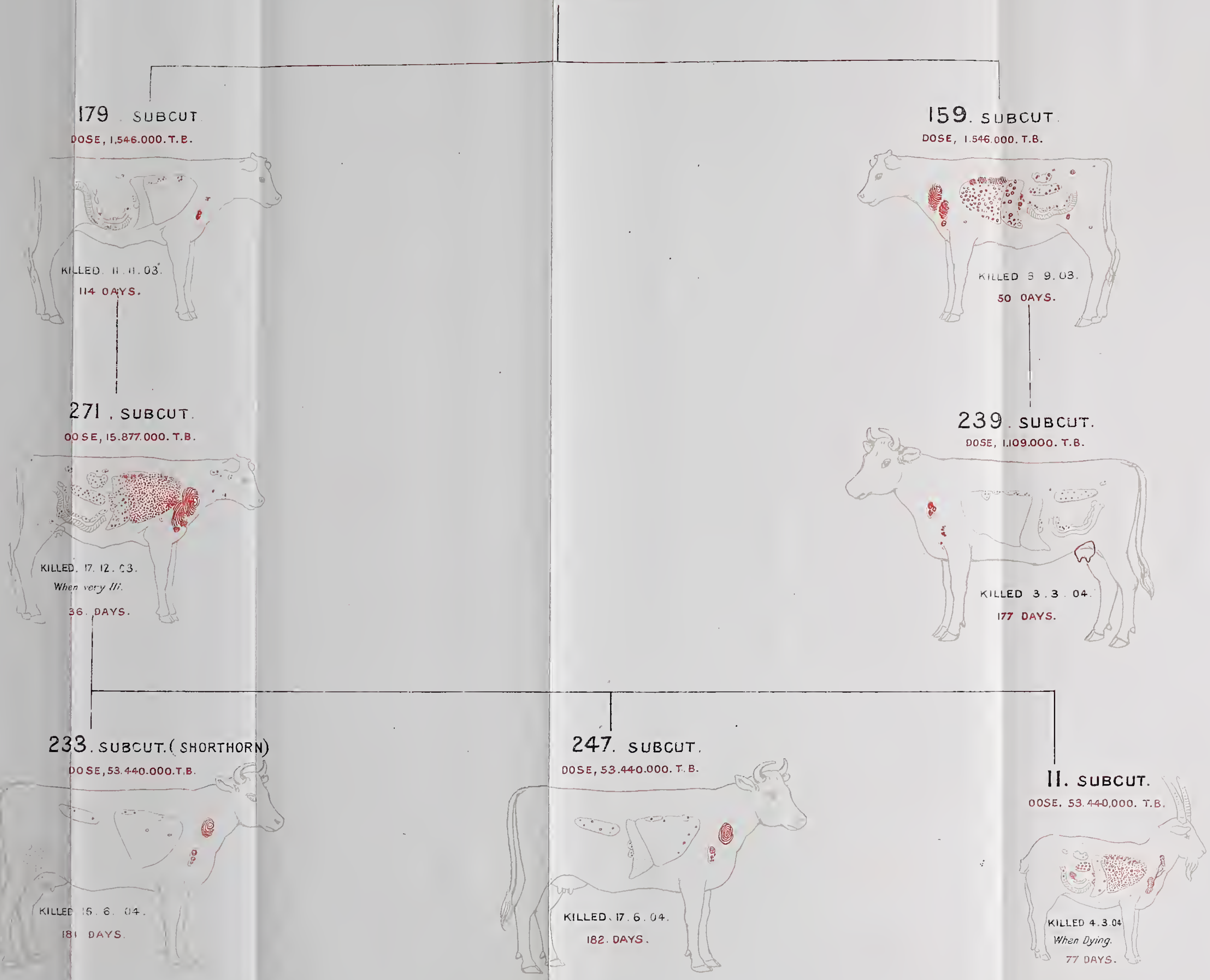
References.—2nd Interim Report : Page 14, paras. 21 to 27 ; page 25, paras. 43 and 44 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 11 and 303 to 332 ; *Chart of Culture Inoculations*, page 304.

Final Report : Pages 9 to 13.

MESENTERIC GLANDS, VIRUS H. 19, "S.W."

JULY 20TH, 1903.



5139.

CHART 5.

VIRUS H. 19. "S.W." Mesenteric Glands. July 20, 1903.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature of case : *Primary Abdominal Tuberculosis.*

Age of patient : 2 years 9 months.

Material used (*post-mortem*) : Mesenteric Glands only.

Explanation of Chart.—The Chart illustrates (1) the inoculation experiments with *emulsion* of the mesenteric glands, undertaken to determine the original virulence of the virus ; and (2) experiments to test the effect of passage from animal to animal, and the virulence for the goat. (In these experiments *emulsions* of tuberculous tissues were alone employed.)

Experiments on calves with *cultures* of this virus, derived from one or other of the "passage" animals, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 14, paras. 21 to 27 ; page 25, paras. 43 and 44 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 11 and 469 to 488 ; *Table of Culture Inoculations*, page 471.

Final Report : Pages 9 to 13.

MESENTERIC GLANDS. VIRUS H.20."F.L."

20TH OCTOBER 1903.

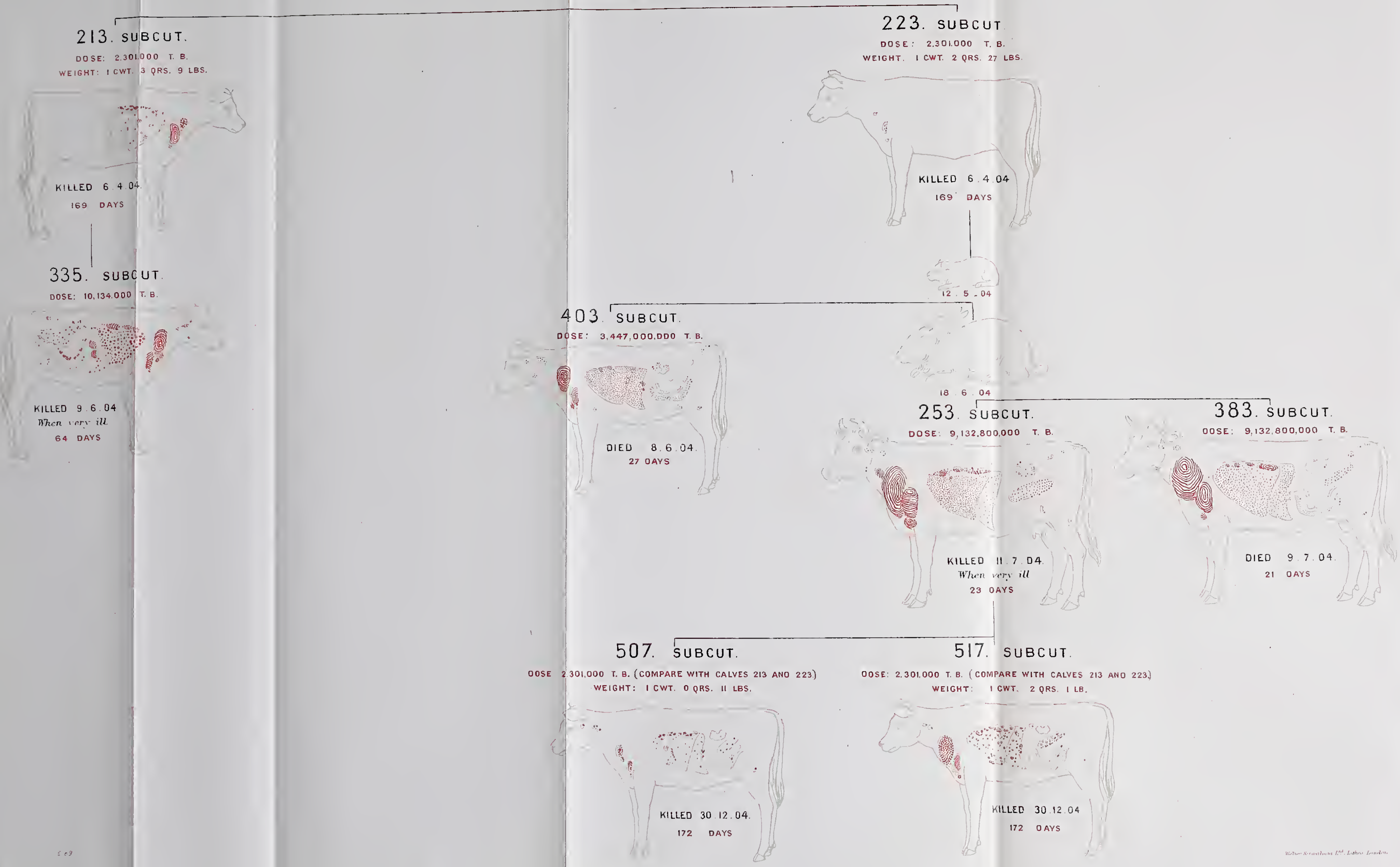


CHART 6.

VIRUS H. 20. "F.L." Mesenteric Glands. October 20, 1903.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature of case: *Primary Abdominal Tuberculosis.*

Age of patient: 2 years, 10 months.

Material used (*post-mortem*) : Mesenteric Glands only.

Explanation of Chart.—The Chart illustrates (1) the inoculation experiments on bovine animals with *emulsion* of the mesenteric glands, made to determine the original virulence of the virus; and (2) experiments to test the effect of passage from animal to animal. (In these experiments *emulsions* of tuberculous tissues alone were employed.)

Experiments on calves with a *culture* of this virus, derived from Calf 213, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report: Page 14, paras. 21 to 27; page 25, paras. 43 and 44; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II: Pages 11 and 489 to 514; *Chart of Culture Inoculations*, page 491.

Final Report: Pages 9 to 13.

CERVICAL GLANDS, VIRUS H. 28. "C.L."

MAY 12TH 1904.

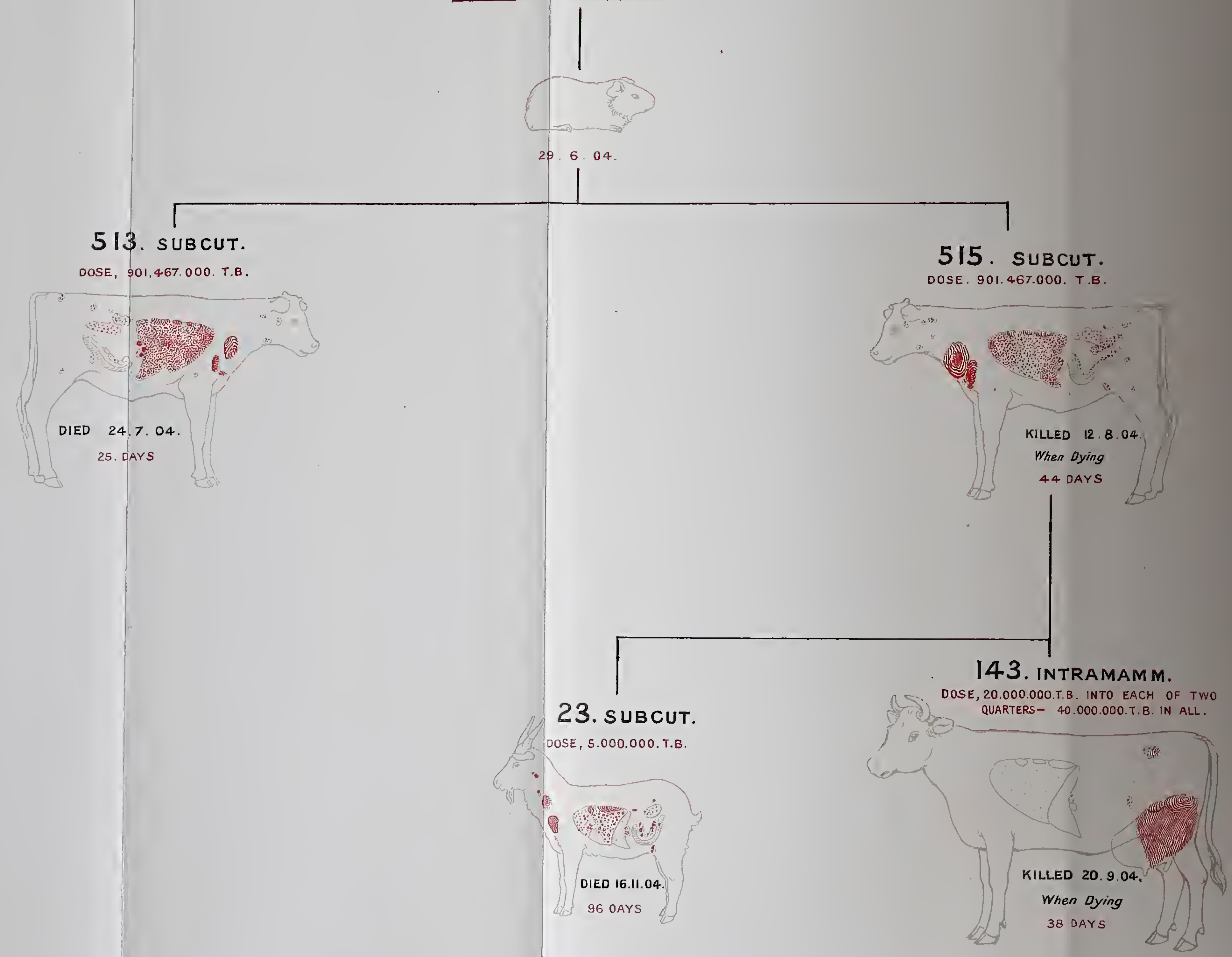


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CHART 7.

VIRUS H. 28. "C.L." Cervical Glands. May 12, 1904.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature of case: *Cervical Gland Tuberculosis.*

Age of patient: 1 year, 2 months.

Material used (*clinical*) : Cervical Glands only.

Explanation of Chart.—The Chart illustrates the inoculation experiments on bovine animals and a goat made to determine the virulence of the virus. An *emulsion* of the original material was inoculated into a guinea-pig, and the tuberculous tissues of the latter used for the inoculation of two calves. An *emulsion* of tuberculous tissue was used also for the subsequent inoculations (cow and goat).

Experiments on calves with a *culture* of the virus derived from the original material were also carried out. (*See* reference in italics.)

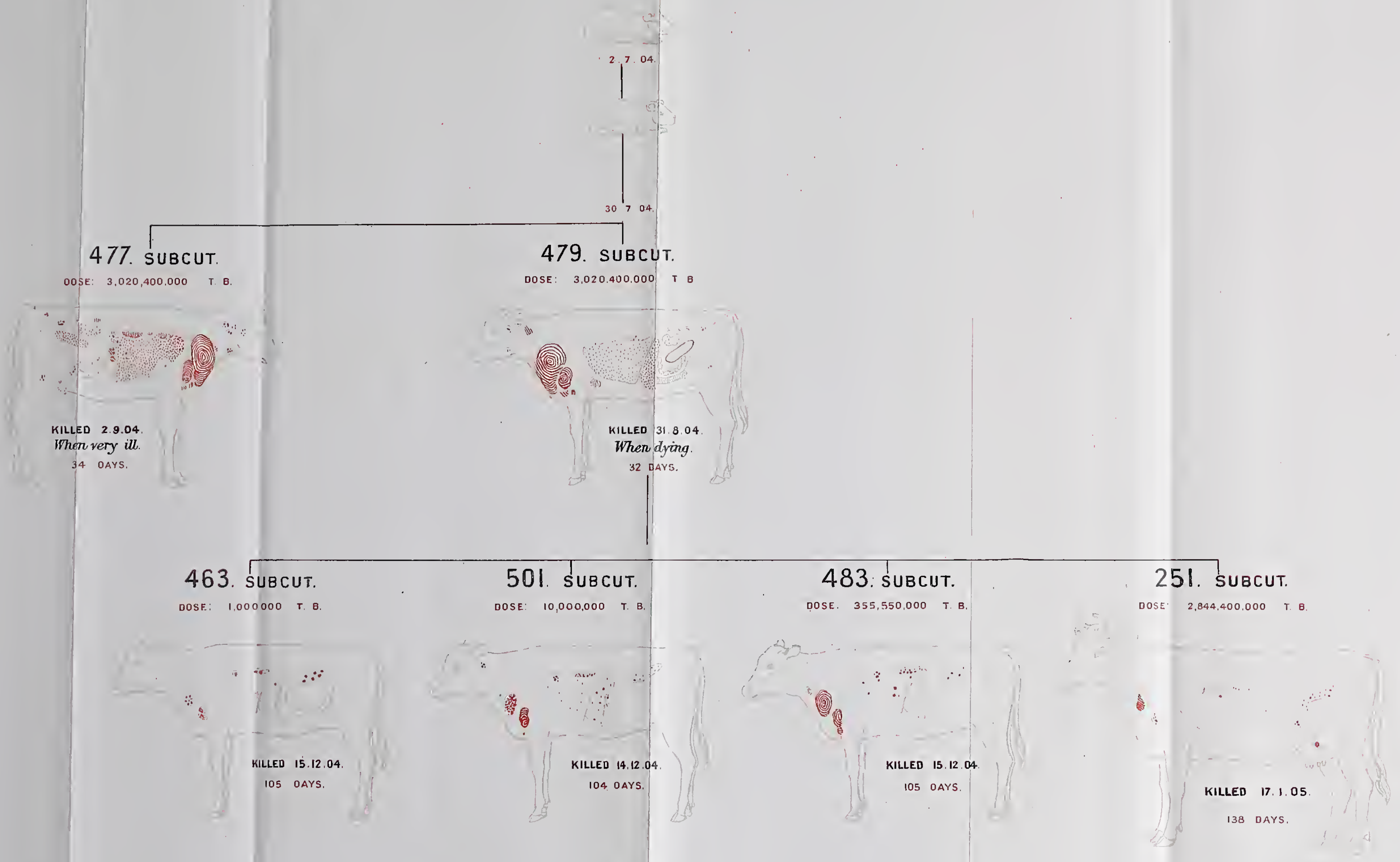
References.—2nd Interim Report: Page 14, paras. 21 to 27 ; page 25, paras. 43 and 44 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 7 and 618 to 647 ; *Charts of Culture Inoculations*, page 620.

Final Report: Pages 9 to 13.

CERVICAL GLANDS VIRUS. H. 29. "M.F."

29TH APRIL, 1904.



5189.

Weller & Graham, Ltd, Lithos. London.

CHART 8.

VIRUS H. 29. "M.F." Cervical Glands. April 29, 1904.

A virus obtained from a case of tuberculosis in man, subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature of case : *Cervical Gland Tuberculosis.*
Age of patient : 7½ years.
Material used (*clinical*) : Cervical Glands only.

Explanation of Chart.—The Chart illustrates the inoculation experiments on bovine animals made to determine the virulence of the virus and the influence of dose. In these experiments *emulsions* of tuberculous tissues alone were employed.

Experiments on calves with a *culture* of the virus, derived from the original material through a guinea-pig, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report: Page 14, paras. 21 to 27; page 25, paras. 43 and 44; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II.: Pages 8 and 648 to 669; *Chart of Culture Inoculations*, page 649.

Final Report: Pages 9 to 13.

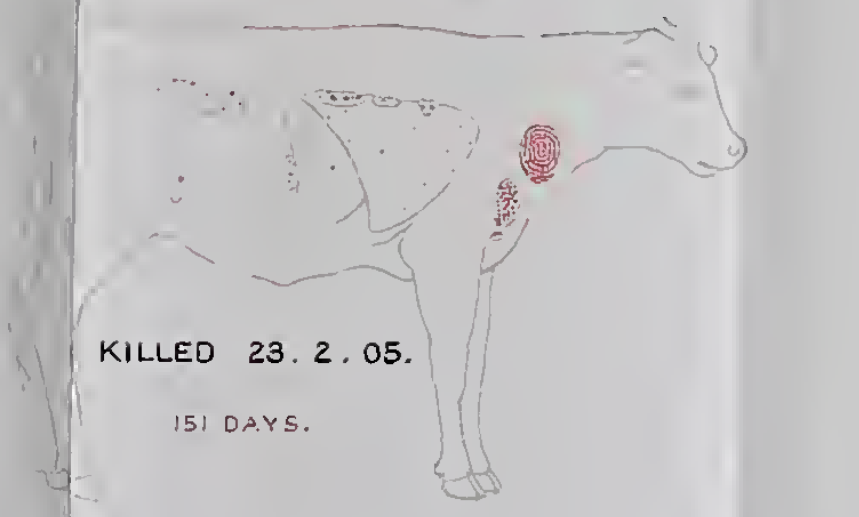
VIRUS. H. 31. "L.F."

CERVICAL GLANDS.

SEPTEMBER, 25TH 1904.

485. SUBCUT.

DOSE. 5.979.000. T.B.

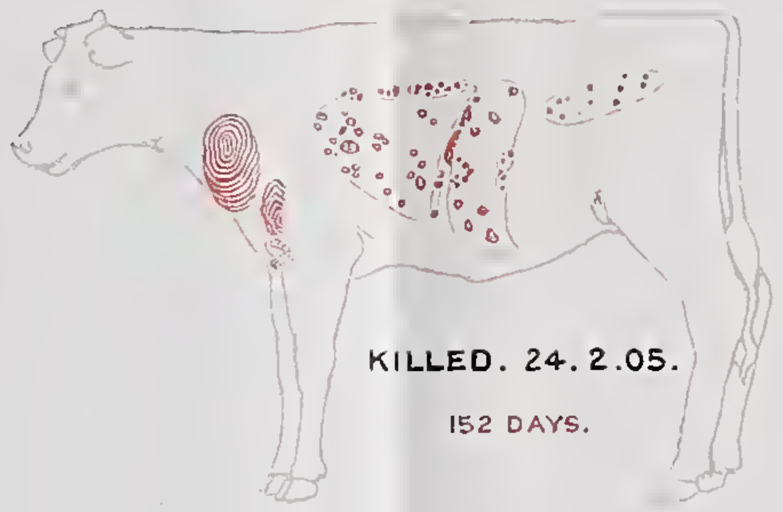


KILLED 23. 2. 05.

151 DAYS.

491. SUBCUT.

DOSE. 5.979:000. T.B.



KILLED. 24. 2. 05.

152 DAYS.

5189.

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CHART 9.

VIRUS H. 31. "L.F." Cervical Glands. September 25, 1904.

A virus obtained from a case of tuberculosis in man, subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature of case: *Cervical Gland Tuberculosis.*
Age of patient: 7 years.
Material used (*clinical*) : Cervical Glands only.

Explanation of Chart.—The Chart illustrates the inoculation experiments on calves with an *emulsion* of the cervical glands made to determine the virulence of the virus.

Experiments on calves with *cultures* of the virus, derived directly from the original material, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 14, paras. 21 to 27 ; page 25, paras. 43 and 44 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 8 and 682 to 703 ; *Chart of Culture Inoculations*, page 685.

Final Report : Pages 9 to 13.

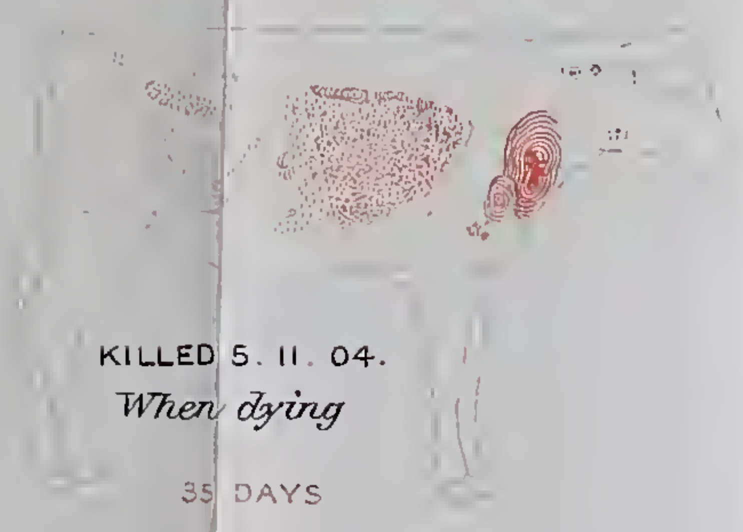
VIRUS H. 32, "Y.W."

OCTOBER 1ST 1904.

LUNG.

533. SUBCUT.

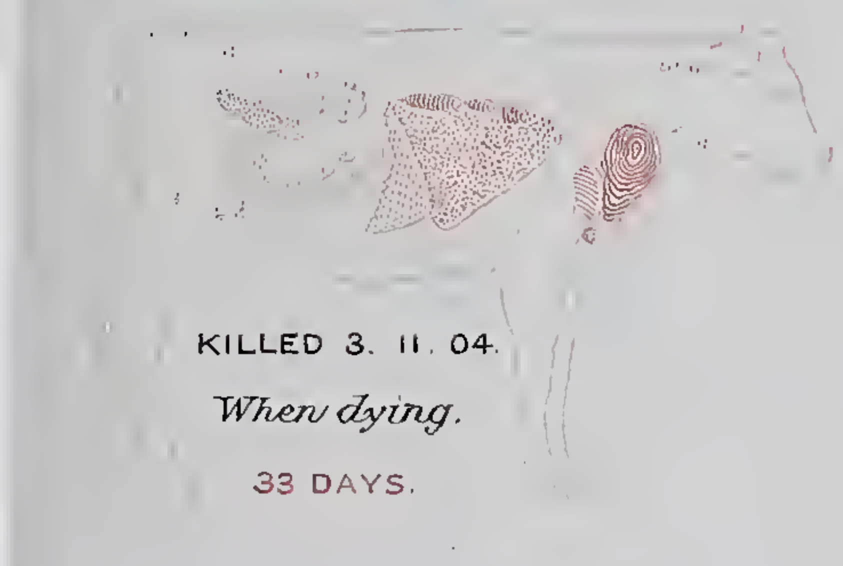
DOSE, 654.160.000. T.B.



KILLED 5. 11. 04.
When dying
35 DAYS

537. SUBCUT.

DOSE, 654.160.000. T.B.

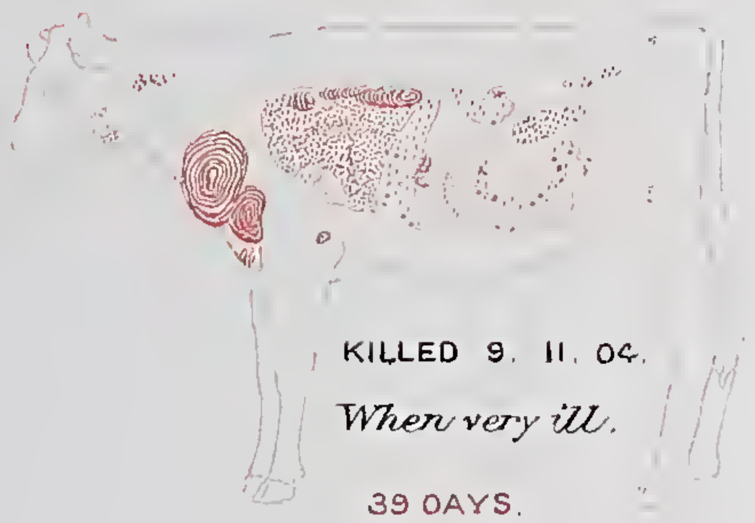


KILLED 3. 11. 04.
When dying.
33 DAYS.

MESENTERIC GLANDS.

465. SUBCUT.

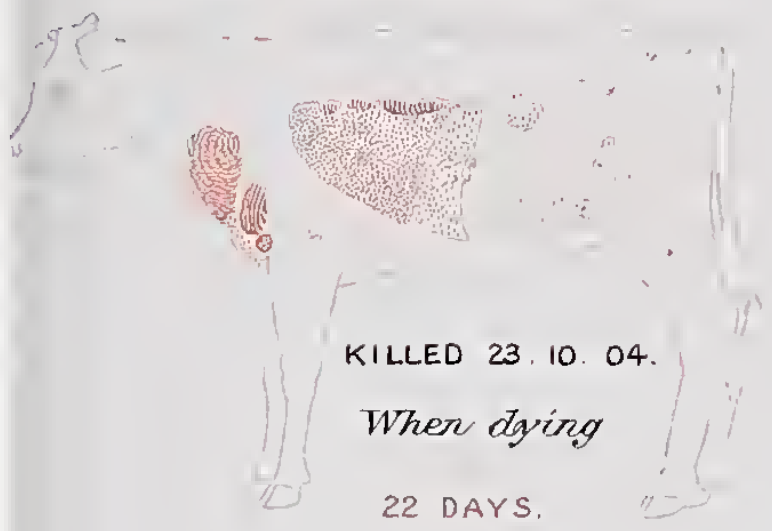
DOSE, 1174.200.000. T.B.



KILLED 9. 11. 04.
When very ill.
39 DAYS.

487. SUBCUT.

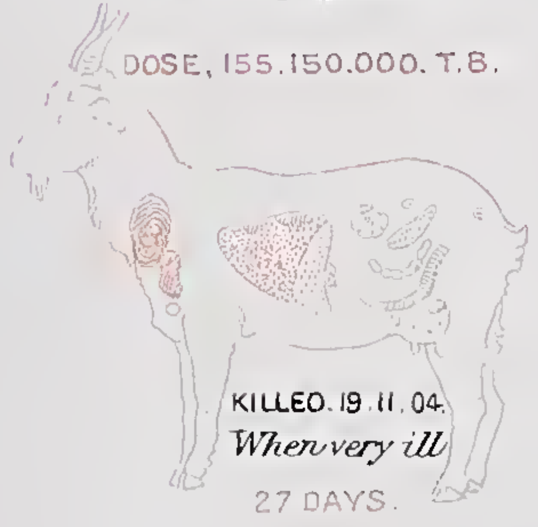
DOSE, 2348.400.000. T.B.



KILLED 23. 10. 04.
When dying
22 DAYS.

7. SUBCUT.

DOSE, 155.150.000. T.B.



KILLED. 19. 11. 04.
When very ill
27 DAYS.

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CHART 10.

VIRUS H. 32. "Y.W." October 1, 1904.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature of case : *Primary Abdominal Tuberculosis.*

Age of patient : 2 years, 9 months.

Material used (*post-mortem*) : Mesenteric Glands and Lung. The Bronchial Glands also were used for experiment (*See* references below.)

Explanation of Chart.—The Chart illustrates the inoculation experiments on calves with *emulsions* of the original material made to determine the virulence of the virus. An emulsion of the lung was tested as well as that of the mesenteric glands, in order to determine whether there was any difference in virulence between the bacilli from these two sources.

The virulence of the virus for the goat was also tested, an *emulsion* of tuberculous tissue from one of the calves being used.

An *emulsion* of the bronchial glands was inoculated into rabbits. (*See* Vol. II. of Appendix.)

Experiments on calves with *cultures* of the virus, derived from the bronchial glands as well as from the mesenteric glands, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 14, paras. 21 to 27 ; page 25, paras. 43 and 44 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 11 and 704 to 725 ; *Chart of Culture Inoculations*, pages 707 and 708.

Final Report : Pages 9 to 13.

VIRUS H.59."L.B."

CULTURE INOCULATIONS

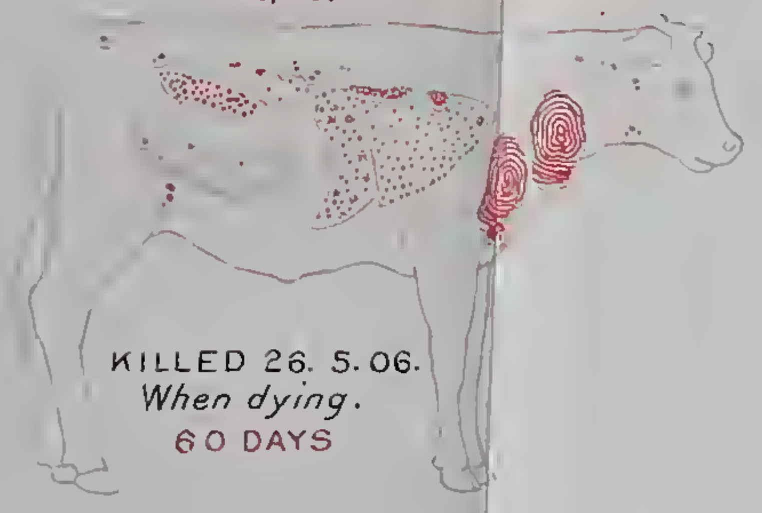
LUNG.

27TH MARCH, 1906.

STRAIN DERIVED FROM HUMAN LUNG, DIRECT.
TESTED FOR VIRULENCE AFTER 7 WEEKS ARTIFICIAL CULTIVATION.
SUBCULTURE ON BOVINE SERUM USED, 20 DAYS OLD.

965. SUBCUT.

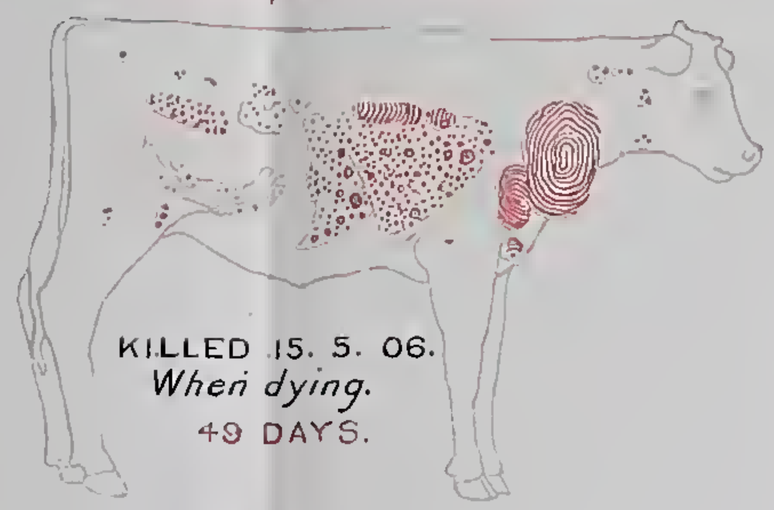
DOSE, 50. MILLIGRAMMES.



KILLED 26. 5. 06.
When dying.
60 DAYS

987. SUBCUT.

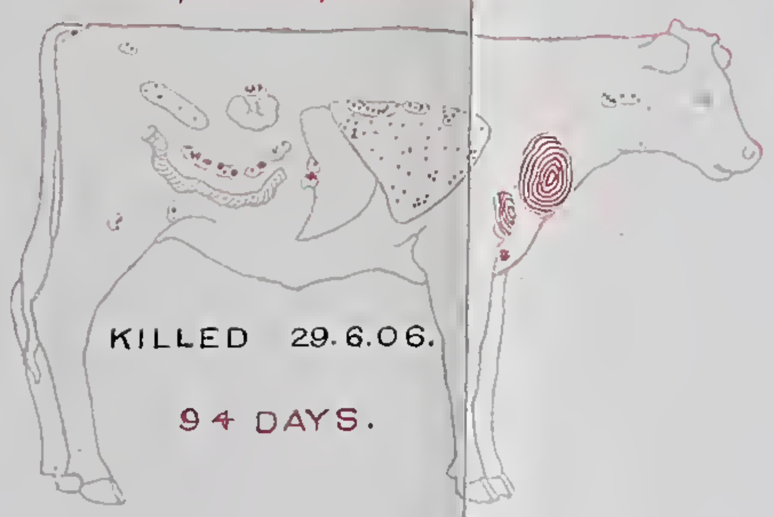
DOSE, 10. MILLIGRAMMES.



KILLED 15. 5. 06.
When dying.
49 DAYS.

991. SUBCUT.

DOSE, 10. MILLIGRAMMES.



KILLED 29.6.06.
94 DAYS.

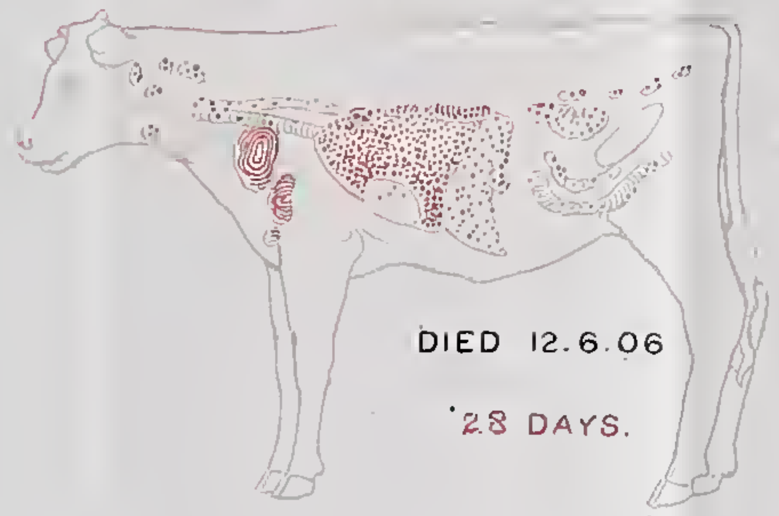
MESENTERIC GLANDS.

15TH MAY, 1906.

STRAIN DERIVED FROM HUMAN MESENTERIC GLANDS, THROUGH G.P. 1849.
TESTED FOR VIRULENCE AFTER 2 MONTHS ARTIFICIAL CULTIVATION.
THIRD SUBCULTURE ON BOVINE SERUM USED, 21 DAYS OLD.

1063. SUBCUT.

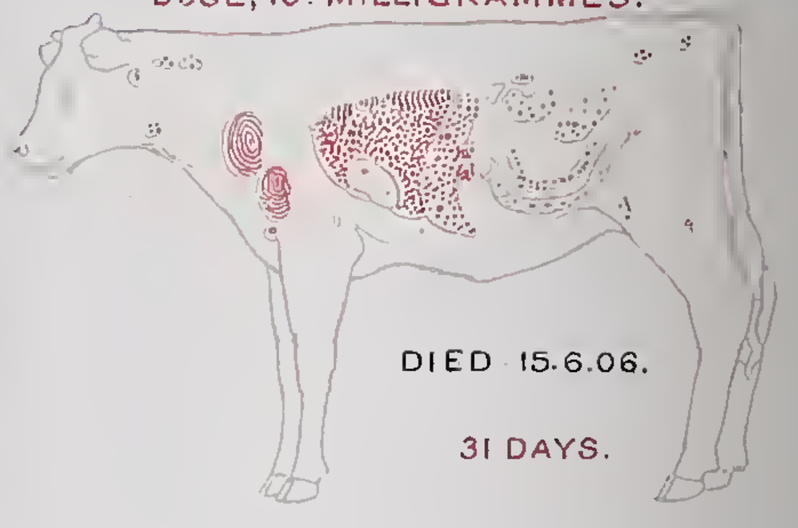
DOSE, 50. MILLIGRAMMES.



DIED 12.6.06
28 DAYS.

1077. SUBCUT.

DOSE, 10. MILLIGRAMMES.



DIED 15.6.06.
31 DAYS.

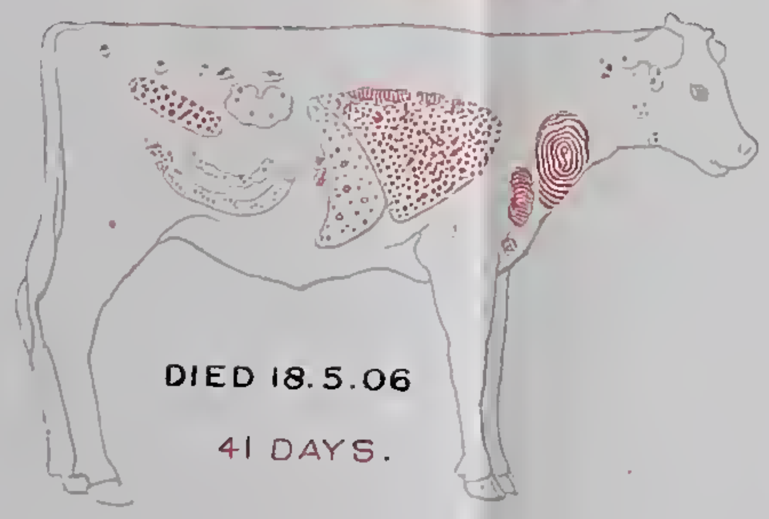
CERVICAL GLANDS.

7TH APRIL, 1906.

STRAIN DERIVED FROM HUMAN CERVICAL GLANDS, DIRECT.
TESTED FOR VIRULENCE AFTER 2 MONTHS ARTIFICIAL CULTIVATION.
SECOND SUBCULTURE ON BOVINE SERUM USED, 21 DAYS OLD.

1037. SUBCUT.

DOSE, 10. MILLIGRAMMES.



DIED 18.5.06
41 DAYS.

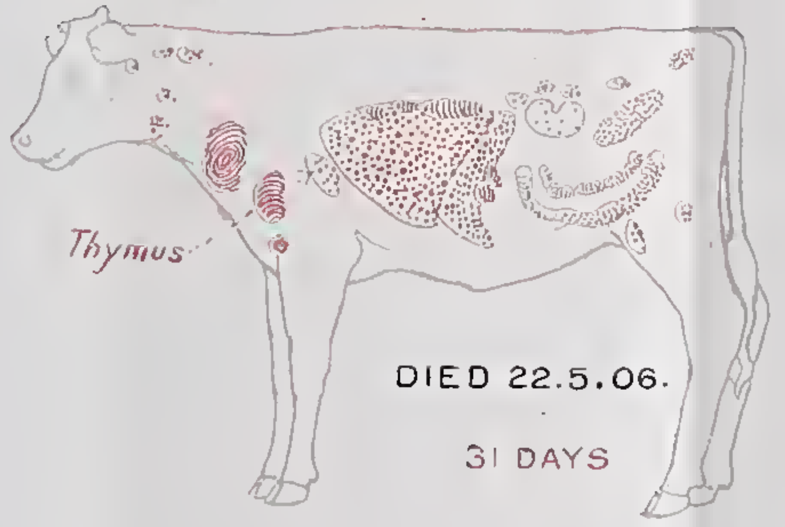
BRAIN.

21ST APRIL, 1906.

STRAIN DERIVED FROM HUMAN BRAIN, THROUGH G.P. 1860.
TESTED FOR VIRULENCE AFTER 2 MONTHS ARTIFICIAL CULTIVATION.
SECOND SUBCULTURE ON BOVINE SERUM USED, 21 DAYS OLD.

1057, SUBCUT.

DOSE, 10. MILLIGRAMMES.



DIED 22.5.06.
31 DAYS

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CHART 11.

VIRUS H. 59. "L.B." Culture Inoculations.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the bovine tubercle bacillus.**

Nature of case: *Primary Abdominal Tuberculosis.*

Age of patient: 2 years, 9 months.

Material used (*post-mortem*) : Mesenteric Glands, Cervical Glands, Lung, and Brain. (Cultures only used.)

Explanation of Chart.—The Chart illustrates the inoculation experiments on calves with several strains of cultures obtained from the original material, made to determine (a) the virulence of virus, and (b) whether any differences in virulence existed between the cultures derived from the several lesions.

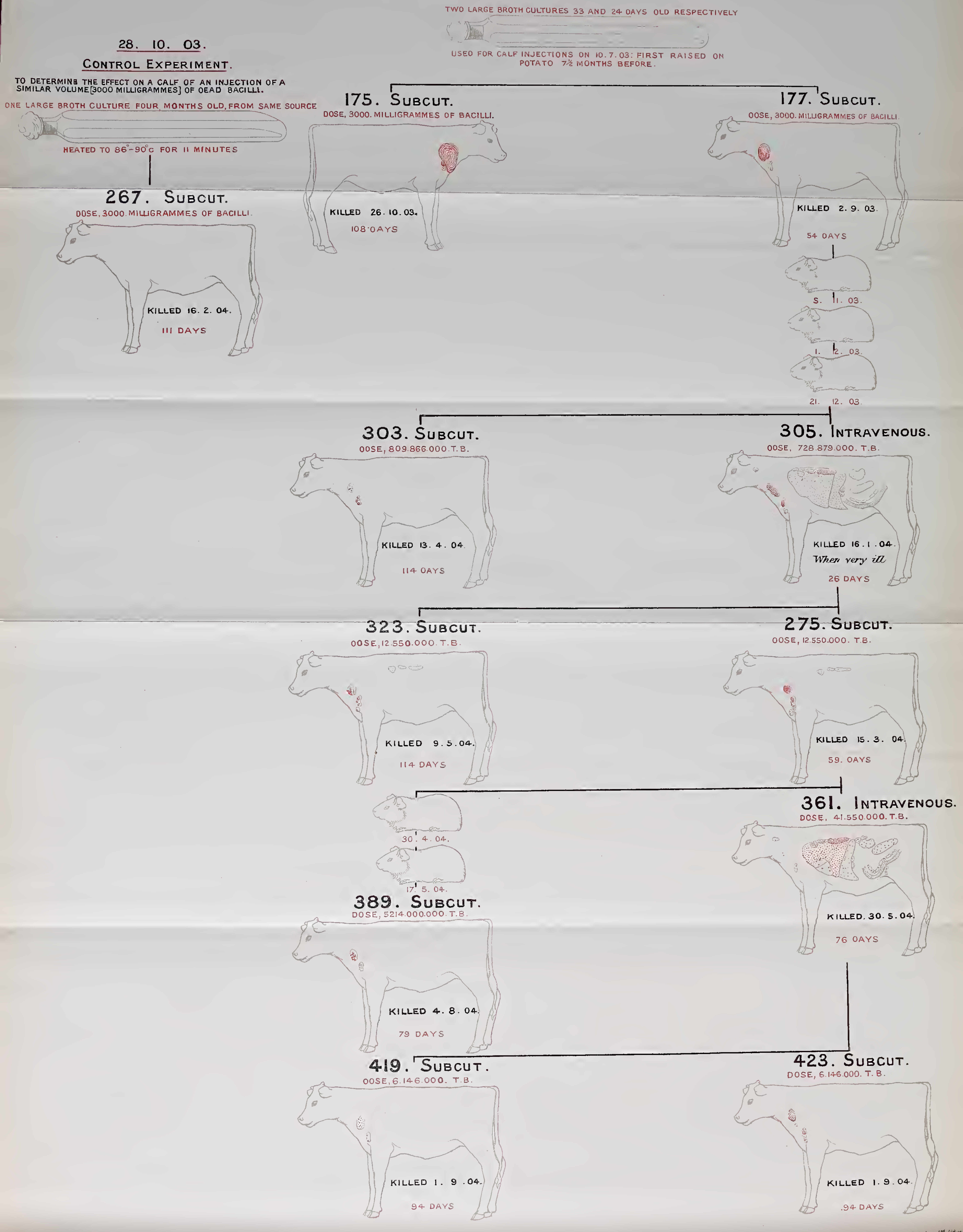
References.—2nd Interim Report: Page 14, paras. 21 to 27 ; page 25, paras 43 and 44 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 12 and 938 to 965.

Final Report : Pages 9 to 13.

MESENTERIC GLANDS, VIRUS H. 8. "S.C."

NOVEMBER 22 1902.



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Weller & Graham, Ltd. Litho.

CHART 12.

VIRUS H. 8. "S.C." Mesenteric Glands. November 22, 1902.

A virus obtained from a case of tuberculosis in man, subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case : *Primary Abdominal Tuberculosis.*

Age of patient : 11 months.

Material used (*post-mortem*) : Mesenteric Glands only.

Explanation of Chart.—The Chart illustrates (1) inoculation experiments on calves with *broth cultures* of the original virus, and (2) experiments—carried on by means of tissue *emulsions*—to test the effect of passage from animal to animal.

Experiments on calves with *serum cultures* also were carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 16, paras. 18 to 33 ; page 26, paras. 45 and 46 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 10 and 159 to 186 ; *Chart of Serum Culture Inoculations*, page 161.

Final Report : Pages 9 to 13.

WRIST-JOINT. VIRUS H.9. "C.T."

11TH DECEMBER, 1902.

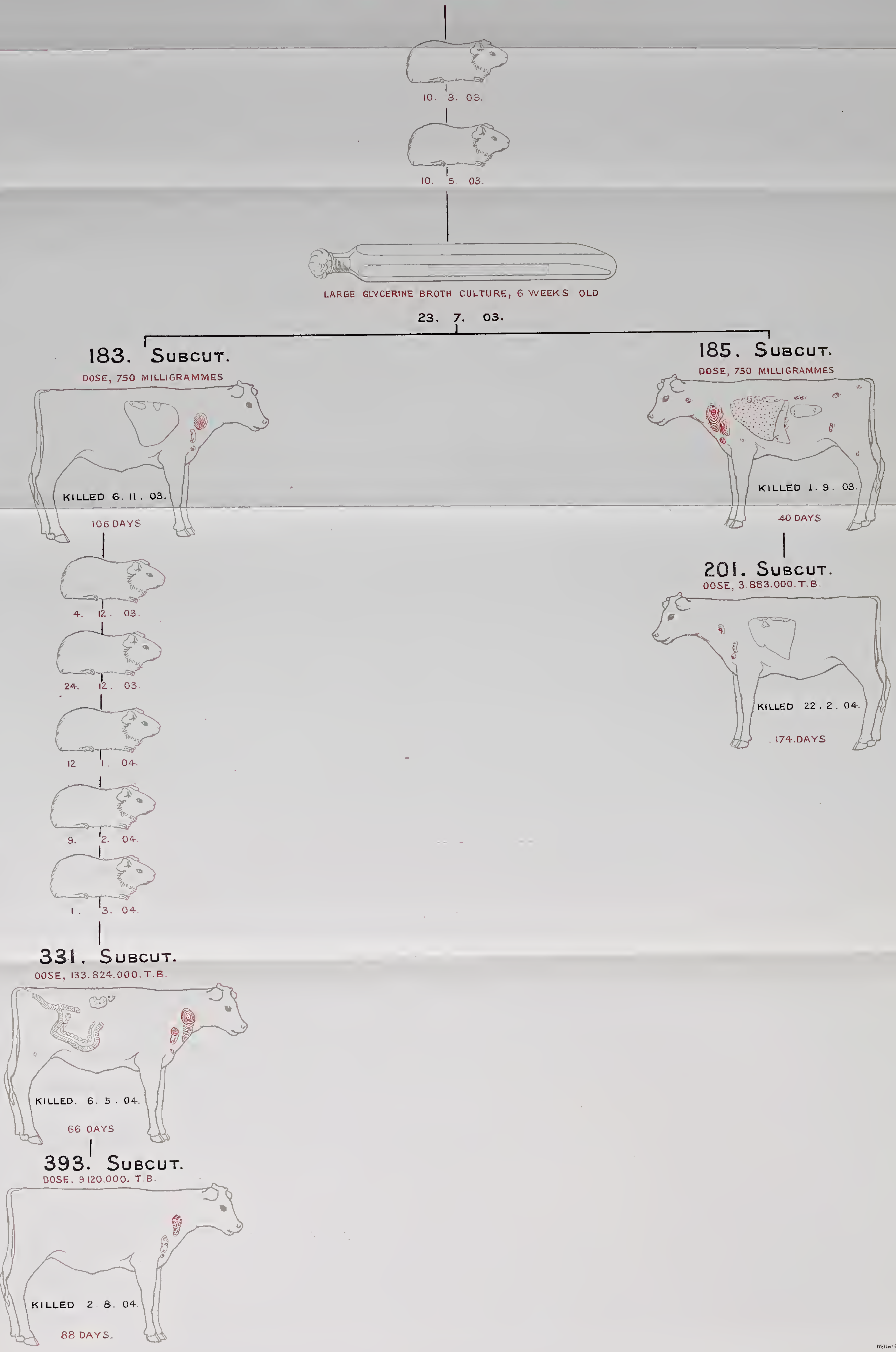

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CHART 13.

VIRUS H. 9. "C.T." Wrist-joint. December 11, 1902.

A virus obtained from a case of tuberculosis in man, subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case : *Joint Tuberculosis.*

Age of patient : 55 years.

Material used (*clinical*) : Tissue removed by operation from wrist-joint.

Explanation of Chart.—The Chart illustrates (1) the inoculation experiments on calves made with *broth culture* (derived from the original material through a guinea-pig) made to determine the original virulence of the virus, and (2) experiments—carried on by means of *emulsions* of tuberculous tissues—to test the effect of passage from animal to animal.

Experiments on calves with *serum cultures* also were carried out. (*See* reference in italics.)

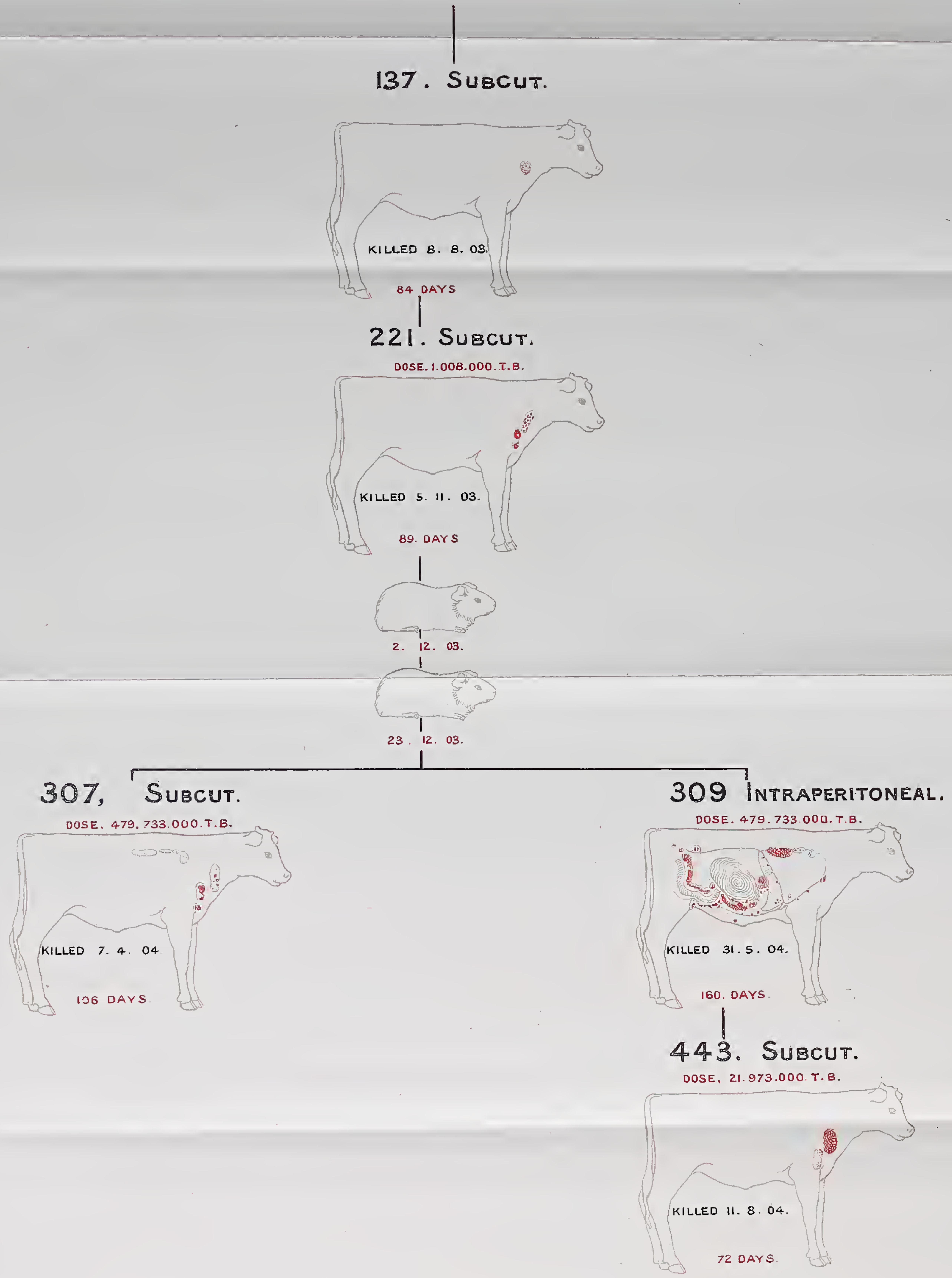
References.—2nd Interim Report: Page 16, paras. 28 to 33 ; page 26, paras. 45 and 46 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 13 and 187 to 200 ; *Chart of Serum Culture Inoculations*, page 189.

Final Report : Pages 9 to 13.

ELBOW-JOINT. VIRUS. H.II. "E.D."

15TH MAY, 1903.



5189.

Weller & Graham Ltd. Litho London.

CHART 14.

VIRUS H. 11. "E.D." Elbow-joint. May 15, 1903

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case : *Joint Tuberculosis.*

Age of patient : 10 years.

Material used (*clinical*) : Synovial membrane of elbow - joint removed by operation.

Explanation of Chart : The Chart illustrates (1) the inoculation experiment on a calf with *emulsion* of the synovial membrane, made to determine the original virulence of the virus and (2) experiments to test the effect of passage from animal to animal. (In these experiments *emulsions* of tuberculous tissues alone were used.)

Experiments on calves with a *culture* of this virus (derived from Calf 309) were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 16, paras. 28 to 33 ; page 26, paras. 45 and 46 ; pages 72 and 97.

Appendix to 2nd Interim Report : Vol. II., pages 13 and 245 to 258 ; *Chart of Culture Inoculations*, page 247.

Final Report : Pages 9 to 13

MESENTERIC GLANDS, VIRUS H.12, "H.N."

23RD MAY, 1903.

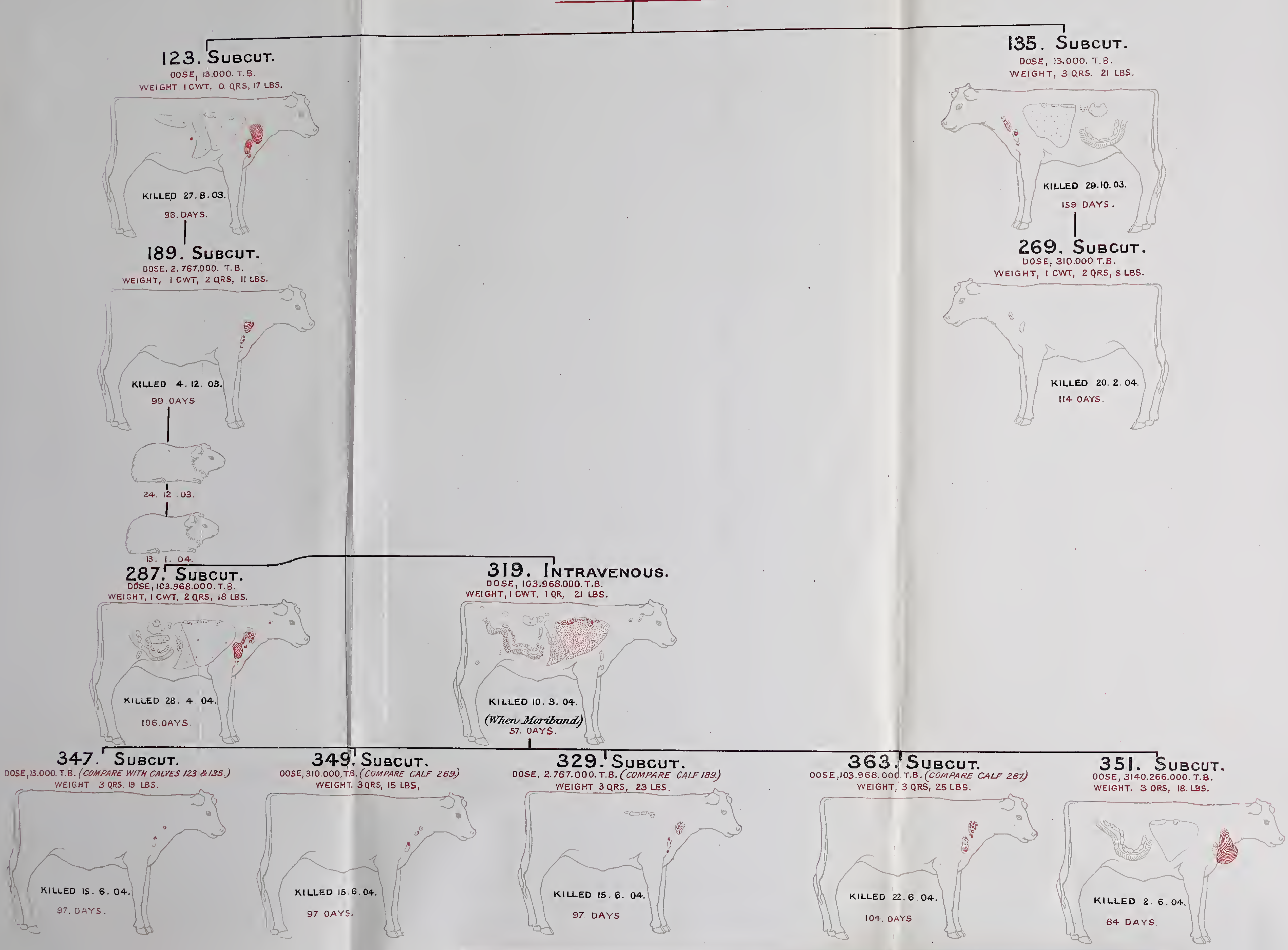


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CHART 15.

VIRUS H. 12. "H.N." Mesenteric Glands. May 23, 1903.

A virus obtained from a case of tuberculosis in man subsequently found to have een caused by **the human tubercle bacillus.**

Nature of case : *Primary Abdominal Tuberculosis.*

Age of patient : 15 years.

Material used (*post-mortem*) : Mesenteric Glands only.

Explanation of Chart.—The Chart illustrates (1) the inoculation experiments on calves with *emulsion* of the mesenteric glands made to determine the original virulence of the virus and (2) experiments to test the effect of passage from animal to animal. (In these experiments *emulsions* of tuberculous tissues alone were employed.)

Experiments on calves with a culture of the virus (derived from Calf 263) were also carried out. (*See* reference in italics.)

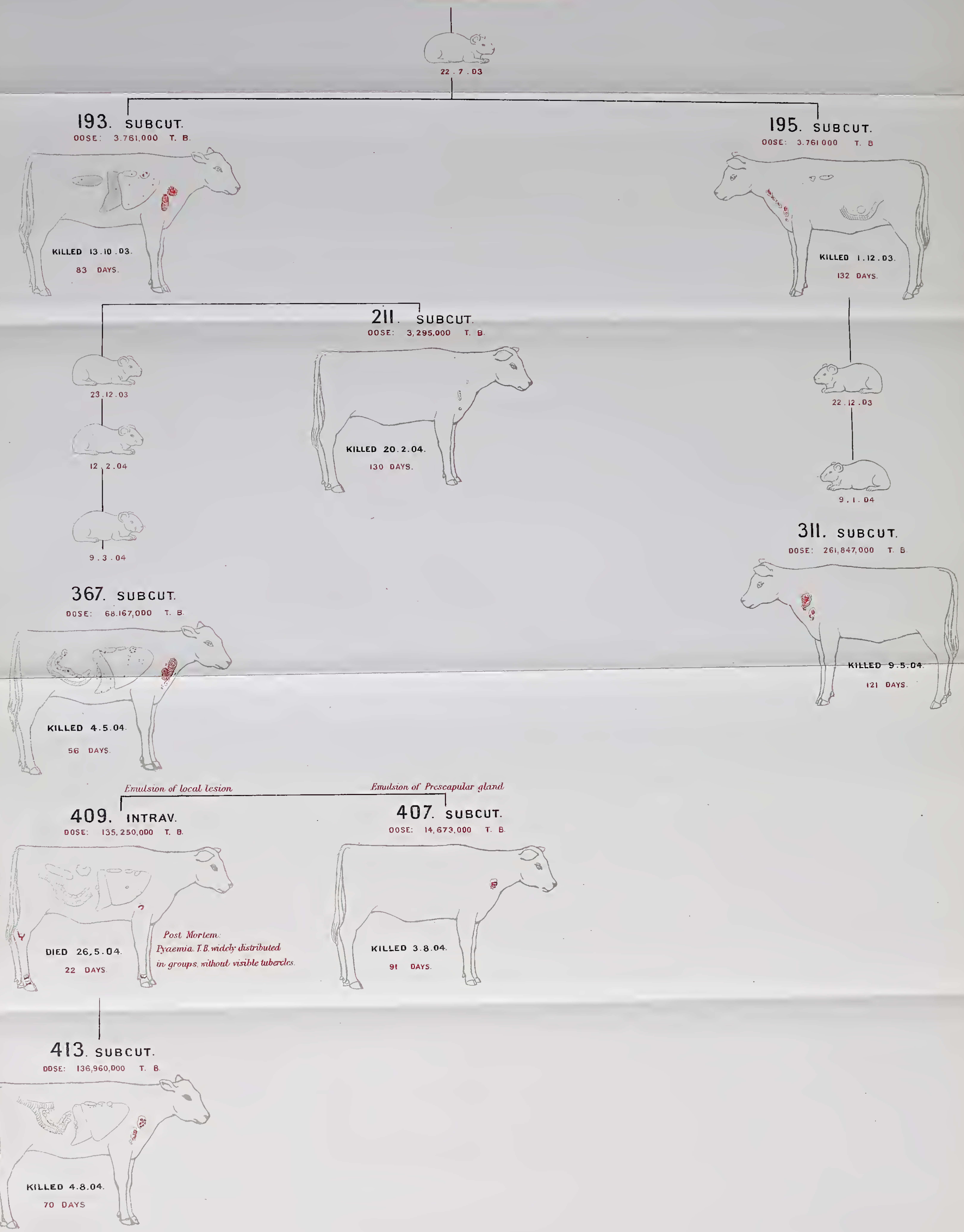
References.—2nd Interim Report : Page 16, paras. 28 to 33 ; page 26, paras. 5 and 46 ; pages 72 and 97.

Appendix to 2nd Interim Report : Vol. II., pages 10 and 259 to 278 ; *Chart f Culture Inoculations*, page 261.

Final Report : Pages 9 to 13.

ANKLE JOINT. VIRUS H.15."I.W."

17TH JUNE 1903.



Post Mortem.
Pyaemia. T.B. widely distributed in groups, without visible tubercles.

5189.

Walker & Graham Ltd, Lithog. London.

CHART 16.

VIRUS H. 15. "I.W." Ankle-joint. June 17, 1903.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case: *Multiple Tuberculous Osteitis.*

Age of patient: 4 years.

Material used (*clinical*): Scrapings from the os calcis and a small gland removed from the left elbow.

Explanation of Chart.—The Chart illustrates (1) the inoculation experiments on calves with an *emulsion* of the scrapings and gland made to determine the original virulence of the virus and (2) experiments to test the effect of passage from animal to animal. (In these experiments *emulsions* of tuberculous tissues were alone employed.)

Experiments on calves with a *culture* of the virus derived from Calf 413, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report: Page 16, paras. 28 to 33; page 26, paras. 45 and 46; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II.: Pages 14 and 333 to 348; *Chart of Culture Inoculations*, page 335.

Final Report: Pages 9 to 13.

MESENTERIC GLANDS VIRUS. H. 18. "T.T."

26TH JUNE, 1903.

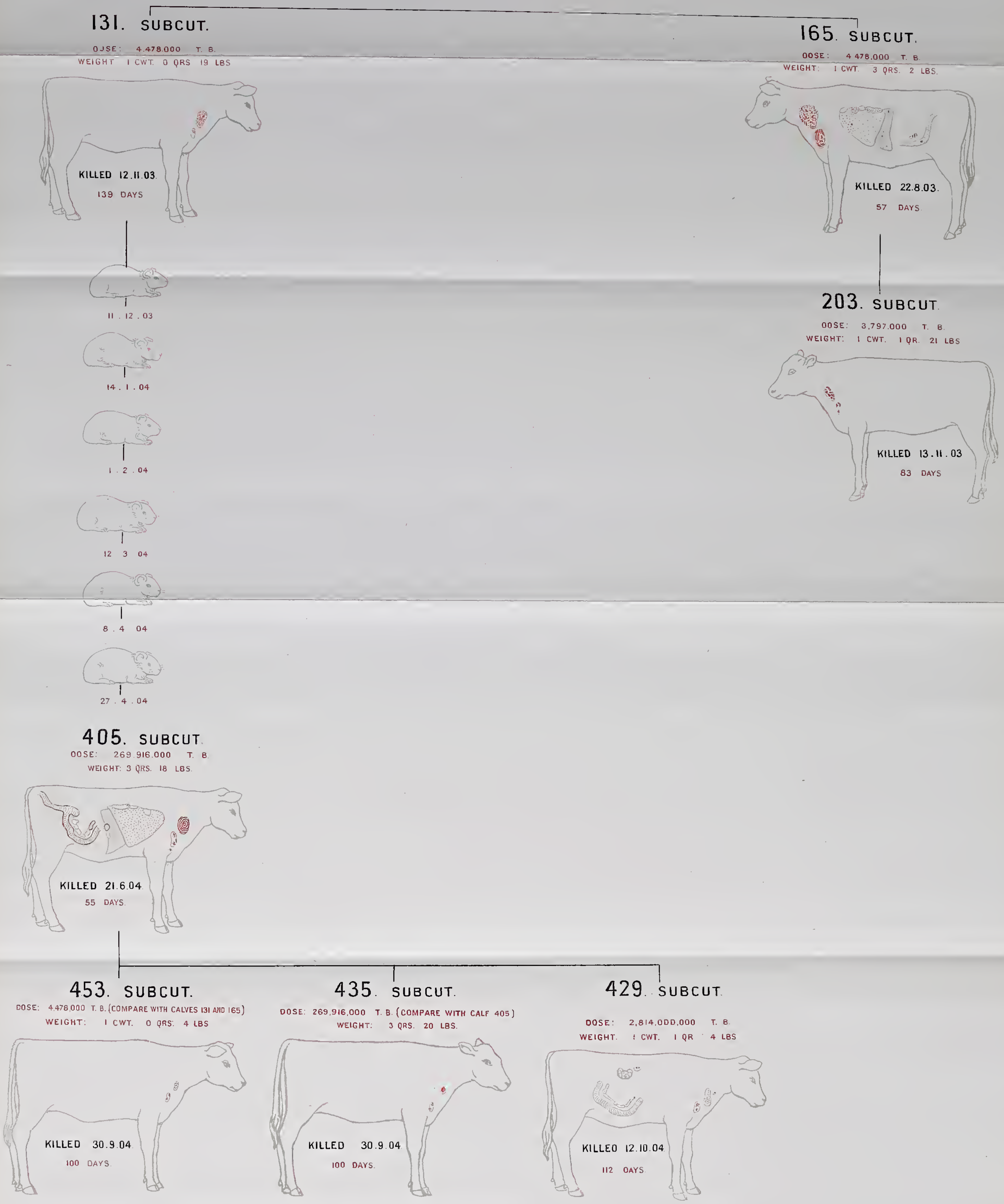


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CHART 17.

VIRUS H. 18. "T.T." Mesenteric Glands. June 26, 1903.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case : *Primary Abdominal Tuberculosis.*

Age of patient : 2 years, 5 years.

Material used (*post-mortem*) : Mesenteric Glands only.

Explanation of Chart.—The Chart illustrates (1) the inoculation experiments on calves with *emulsion* of the mesenteric glands made to determine the original virulence of the virus and (2) experiments to test the effect of passage from animal to animal. (In these experiments *emulsions* of tuberculous tissues alone were employed.)

Experiments on calves with a *culture* of the virus derived from Calf 405, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 16, paras. 28 to 33 ; page 26, paras. 45 and 46 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II : Pages 11 and 453 to 468 ; *Chart of Culture Inoculations*, page 455.

Final Report : Pages 9 to 13.

VIRUS H. 22, "F.W."

DECEMBER, 5.1903.

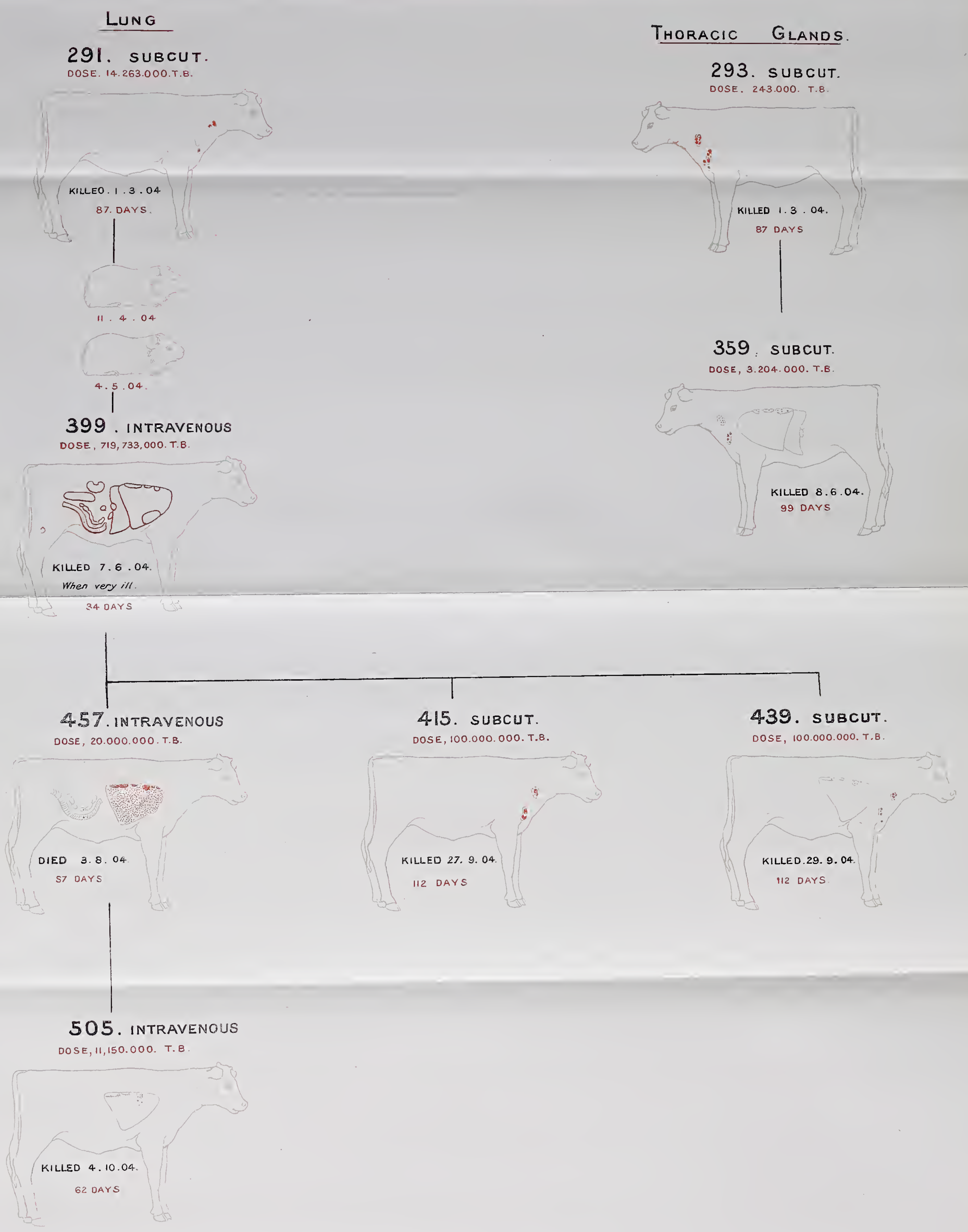

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CHART 18.

VIRUS H. 22. "F.W." Lung, and Thoracic Glands. December 5, 1903.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case : *Pulmonary Tuberculosis.*

Age of patient : 16 years.

Material used (*post-mortem*) : Lung, and Thoracic Glands only.

Explanation of Chart.—The Chart illustrates (1) the inoculation experiments on calves with an *emulsion* of lung and an *emulsion* of thoracic glands, made to determine the original virulence of the virus ; and (2) experiments to test the effect of passage from animal to animal. (In these experiments *emulsions* of tuberculous tissues alone were employed.)

Experiments on calves with a *culture* of the virus (derived from Calf 293) were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 16, paras. 28 to 33 ; page 26, paras. 45 and 46 ; pages 72 and 79.

Appendix to 2nd Interim Report, Vol. II : Pages 4 and 531 to 552 ; *Chart of Culture Inoculations*, page 533.

Final Report : Pages 9 to 13.

LUNG. VIRUS H. 23. "J.P."

19TH MARCH, 1904.

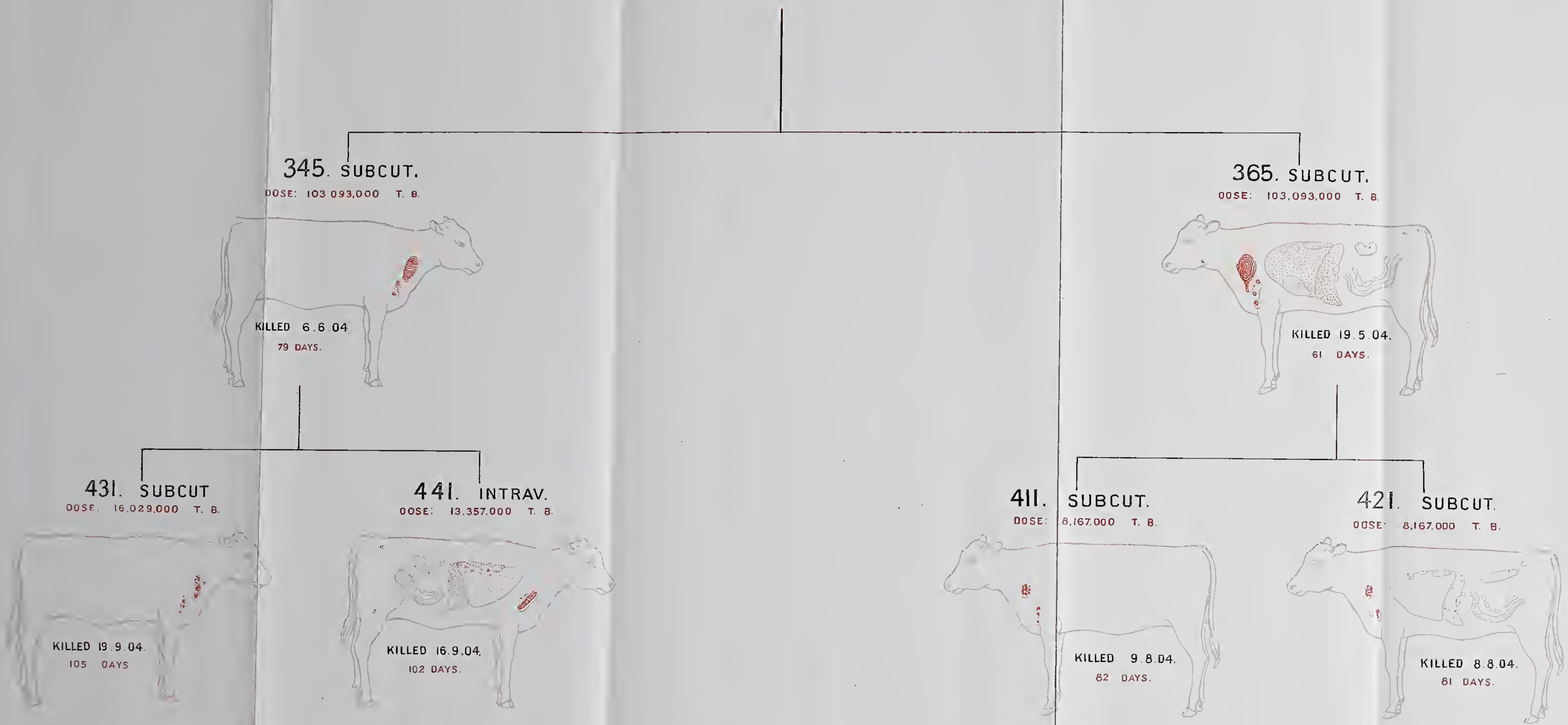

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CHART 19.

VIRUS H. 23. "J.P." Lung. March 13, 1904.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case : *Pulmonary Tuberculosis.*

Age of patient : 41 years.

Material used (*post-mortem*) : Lung only.

Explanation of Chart.—The Chart illustrates (1) the inoculation experiments on calves with *emulsion* of the lung made to determine the original virulence of the virus ; and (2) experiments to test the effect of passage from animal to animal. (In these experiments *emulsions* of tuberculous tissues alone were employed.

Experiments on calves with a *culture* of the virus (derived from Calf 365) were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 16, paras. 28 to 33 ; page 26, paras. 45 and 46 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 5 and 553 to 568 ; *Chart of Culture Inoculations*, page 556.

Final Report : Pages 9 to 13.

LUNG, VIRUS H. 25. "A.T."

17TH MAY, 1904.

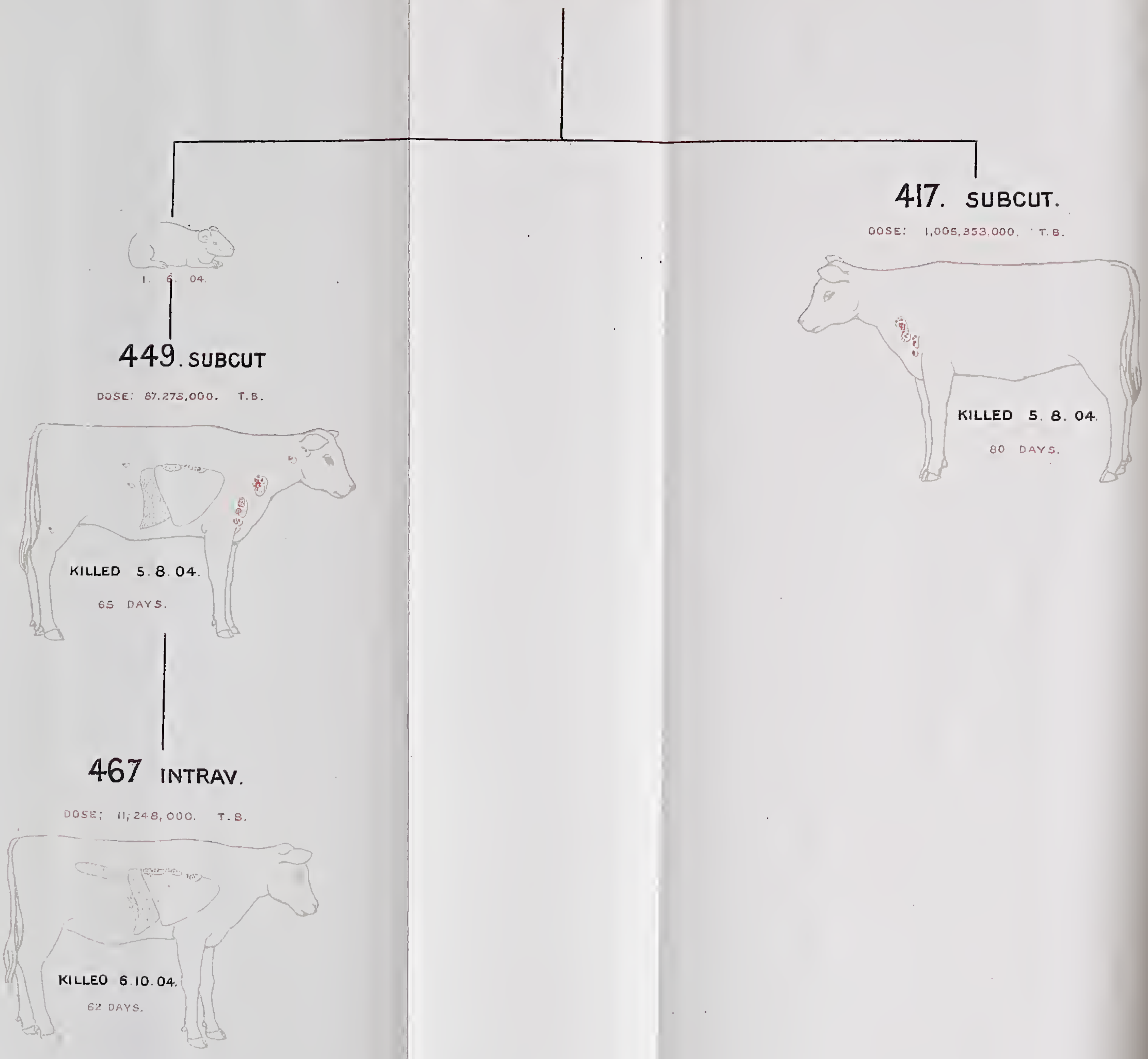

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CHART 20.

VIRUS H. 25. "A.T." Lung. May 17, 1904.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case : *Pulmonary Tuberculosis.*
Age of patient : 30 years.
Material used (*post-mortem*) : Lung only.

Explanation of Chart.—The Chart illustrates the inoculation experiments on calves made to determine the virulence of the virus. (In these experiments *emulsions* of tuberculous tissues alone were employed.)

Experiments with a *culture* of the virus (derived from Calf 417) were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 16, paras. 28 to 33 ; page 26, paras. 45 and 46 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 5 and 575 to 594 ; *Chart of Culture Inoculations*, page 578.

Final Report : Pages 9 to 13.

KIDNEY. VIRUS H. 26 "K.M."

22ND JUNE, 1904.

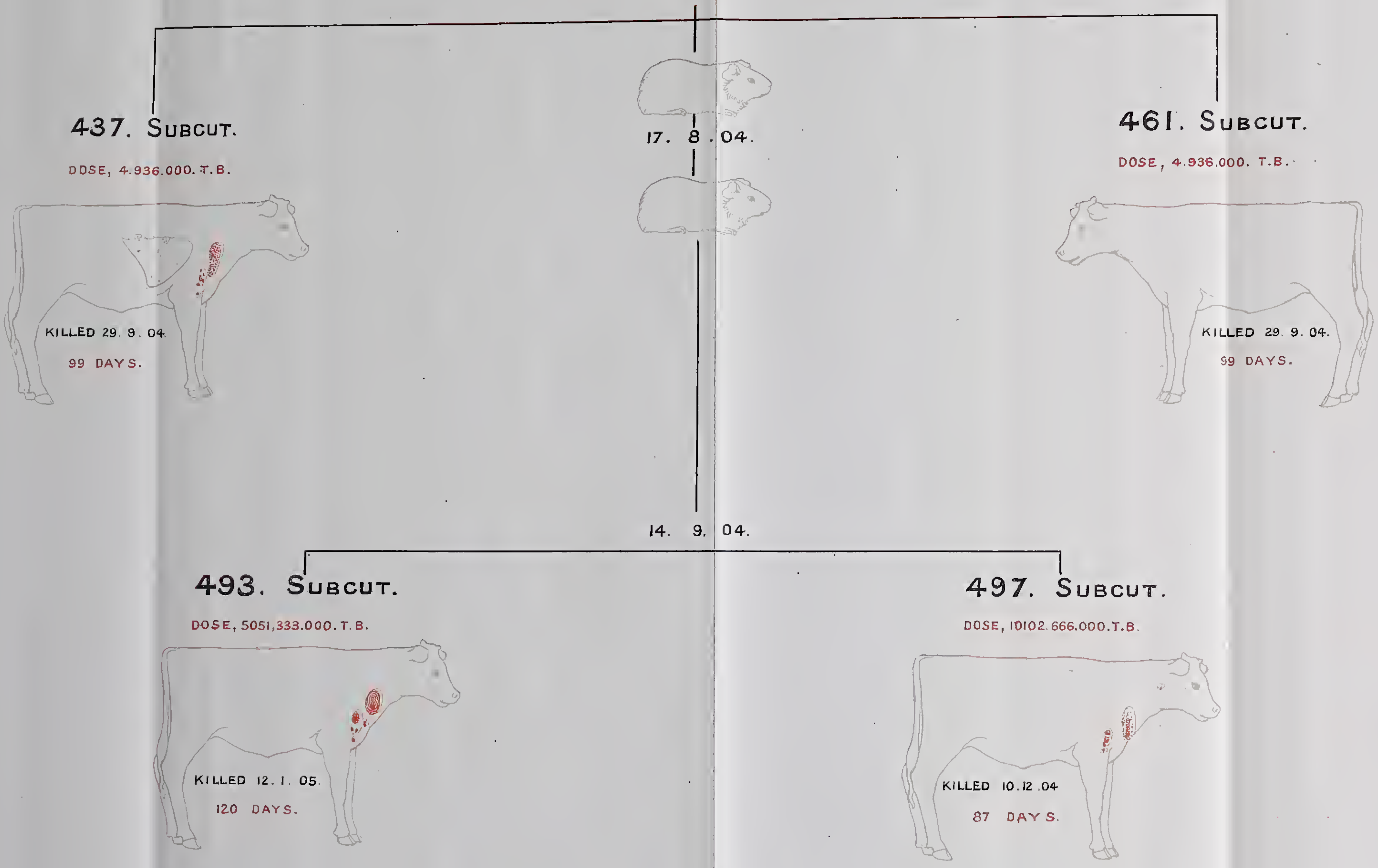

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CHART 21.

VIRUS H. 26. "K.M." Kidney. June 22, 1904.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case: *Tuberculosis of Kidney.*
Age of patient: 25 years.
Material used (*clinical*): Kidney only.

Explanation of Chart.—The Chart illustrates the inoculation experiments on calves made to determine the virulence of the virus. In these experiments *emulsions* of tuberculous tissues alone were employed.

Experiments on calves with a *culture* of the virus (derived from the kidney through a guinea-pig) were also carried out. (*See* reference in italics.)

References.—2nd Interim Report: Page 16, paras. 28 to 33; page 26, paras. 45 and 46; pages 72 and 97.

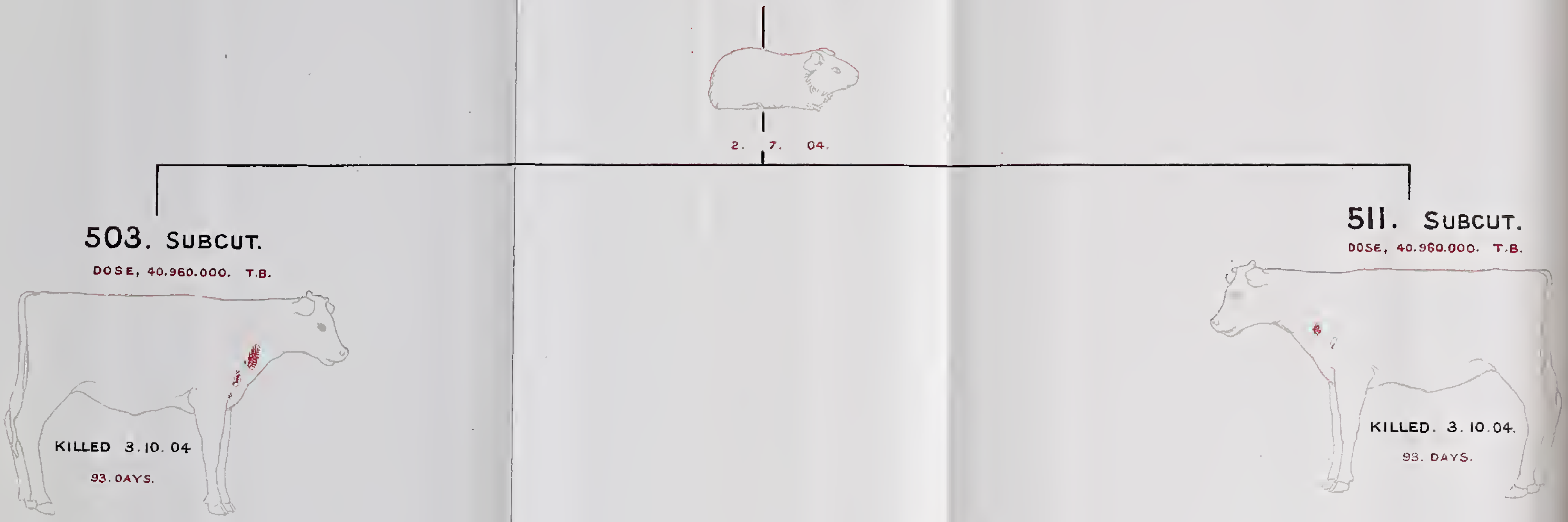
Appendix to 2nd Interim Report, Vol. II.: Pages 15 and 595 to 606; *Chart of Culture Inoculations*, page 598.

Final Report: Pages 9 to 13.

VIRUS H. 27 "B.D."

CERVICAL GLANDS.

APRIL, 21ST 1904.



5189.

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CHART 22.

VIRUS H. 27. "B.D." Cervical Glands. April 21, 1904.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case : *Cervical Gland Tuberculosis.*

Age of patient : 11½ years.

Material used (*clinical*) : Cervical Glands only.

Explanation of Chart.—The Chart illustrates the inoculation experiments on calves made to determine the virulence of the virus ; in these experiments *emulsions* of tuberculous tissues alone were employed.

Experiments on calves with a *culture* of the virus, derived from the original material, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 16, paras. 28 to 33 ; page 26, paras. 45 and 46 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 7 and 607 to 617 ; *Chart of Culture Inoculations*, page 610.

Final Report : Pages 9 to 13.

VIRUS H. 30. "E.M."

MESENTERIC & BRONCHIAL GLANDS

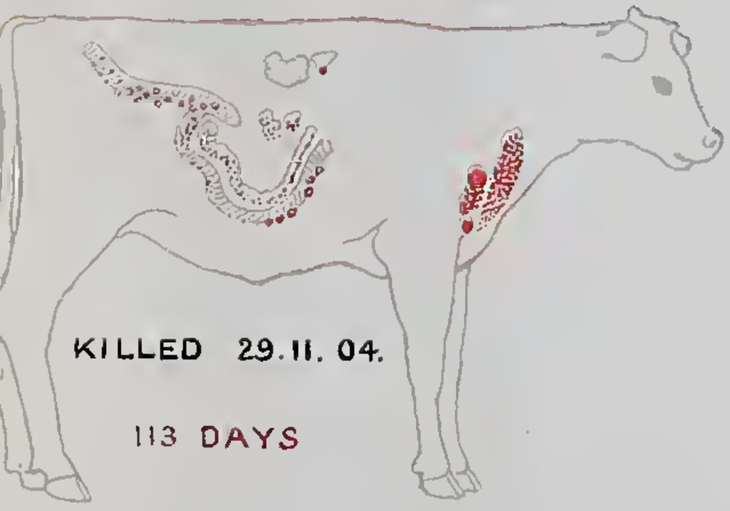
MAY 24TH 1904.

13. 7. 04.

8. 8. 04.

473. SUBCUT.

DOSE, 4.756.800.000.T.B.

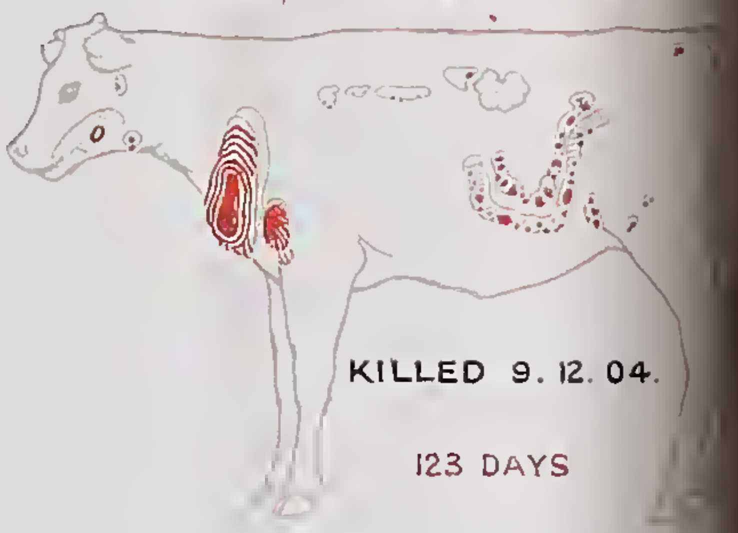


KILLED 29.11.04.

113 DAYS

481 SUBCUT.

DOSE, 4.756.800.000.T.B.



KILLED 9.12.04.

123 DAYS

CHART 23.

VIRUS H. 30. "E.M." Mesenteric and Bronchial Glands. May 24, 1904.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case: *Primary Abdominal Tuberculosis.*

Age of patient: 3 years, 10 months.

Material used (*post-mortem*) : Mesenteric and Bronchial Glands only.

Explanation of Chart.—The Chart illustrates the inoculation experiments on calves made to determine the virulence of the virus. A mixed *emulsion* of the human tissues was used for the inoculation of the first guinea-pig, and *emulsions* of tuberculous tissues were used for infecting the second guinea-pig as well as for the calves.

Experiments on calves with a *culture* of the virus, derived from the original material through a guinea-pig, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report: Page 16, paras. 28 to 33; page 26, paras. 45 and 46; pages 72 and 97.

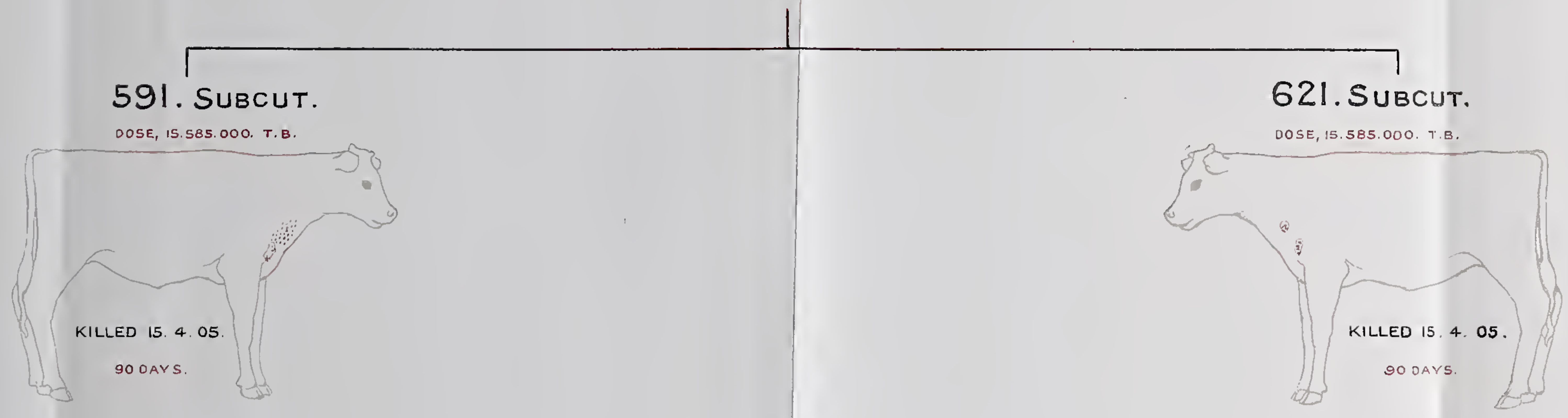
Appendix to 2nd Interim Report, Vol. II. : Pages 11 and 670 to 681 ; *Chart of Culture Inoculations*, page 673.

Final Report : Pages 9 to 13.

VIRUS H. 36. "M.D."

BRONCHIAL GLANDS.

JANUARY, 11TH 1905.



5189.

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CHART 23.

VIRUS H. 30. "E.M." Mesenteric and Bronchial Glands. May 24, 1904.

A virus obtained from a case of tuberculosis in man subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case: *Primary Abdominal Tuberculosis.*

Age of patient: 3 years, 10 months.

Material used (*post-mortem*) : Mesenteric and Bronchial Glands only.

Explanation of Chart.—The Chart illustrates the inoculation experiments on calves made to determine the virulence of the virus. A mixed *emulsion* of the human tissues was used for the inoculation of the first guinea-pig, and *emulsions* of tuberculous tissues were used for infecting the second guinea-pig as well as for the calves.

Experiments on calves with a *culture* of the virus, derived from the original material through a guinea-pig, were also carried out. (*See* reference in italics.)

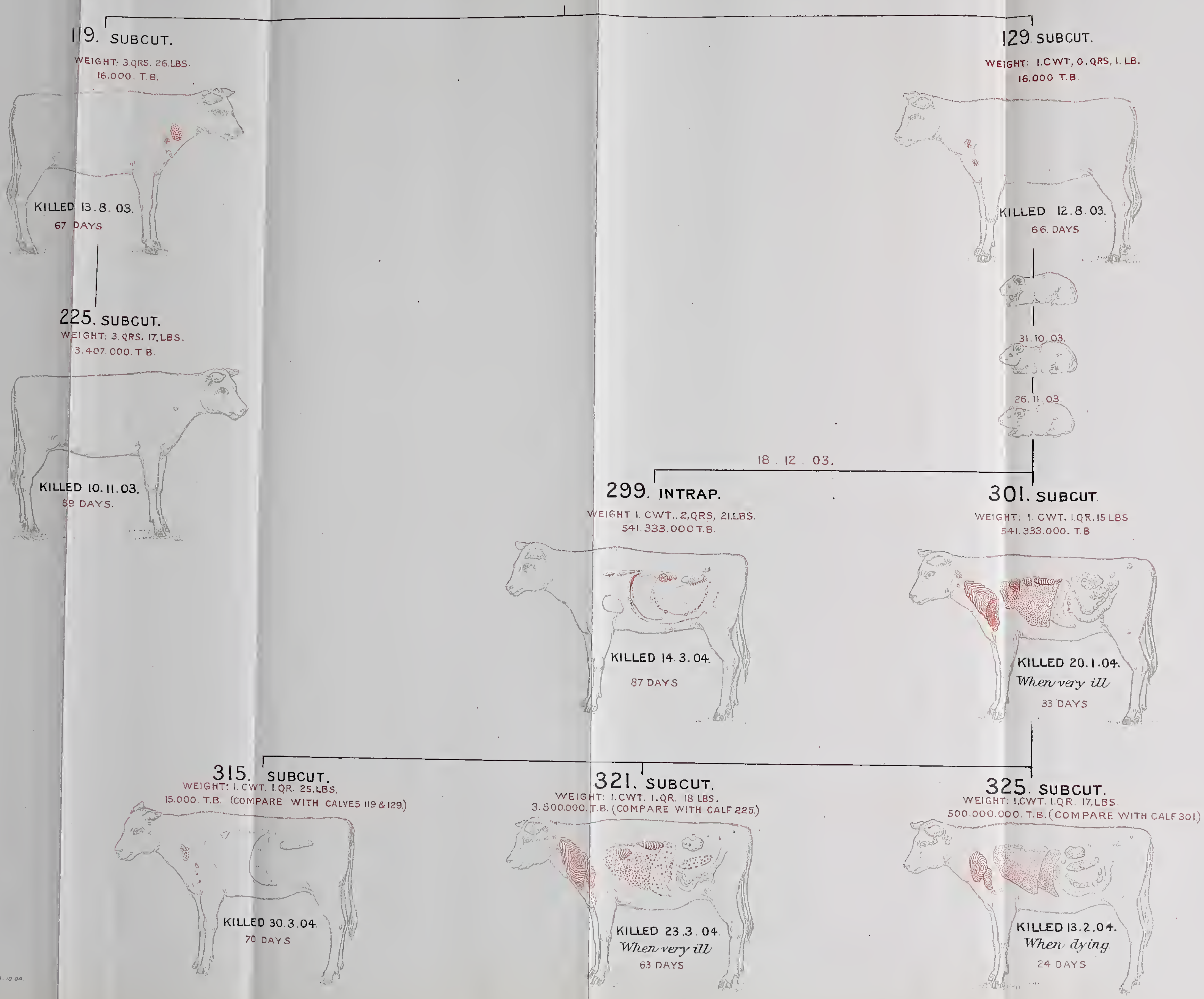
References.—2nd Interim Report: Page 16, paras. 28 to 33; page 26, paras. 45 and 46; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II.: Pages 11 and 670 to 681; *Chart of Culture Inoculations*, page 673.

Final Report: Pages 9 to 13.

BRONCHIAL GLANDS AND SPLEEN. VIRUS. H.13. "A.D."

7TH JUNE 1903.



5169. 10.04.

Weller & Graham, Ltd. Lithos. London

CHART 24.

VIRUS H. 36. "M.D." Bronchial Glands. January 11, 1905.

A virus obtained from a case of tuberculosis in man, subsequently found to have been caused by **the human tubercle bacillus.**

Nature of case : *Primary Abdominal Tuberculosis.*

Age of patient : 4½ years.

Material used for investigation (*post-mortem*) : Bronchial Glands. The Mesenteric Glands also were used, for culture inoculations only. (*See* below.)

Explanation of Chart.—The Chart illustrates the inoculation experiments on calves with an *emulsion* of the bronchial glands made to determine the virulence of the virus.

Experiments with a *culture* of the virus, derived directly from the human mesenteric glands, were also carried out. (*See* reference in italics.)

References.—2nd Interim Report : Page 16, paras. 29 to 33 ; page 26, paras. 45 and 46 ; pages 72 and 97.

Appendix to 2nd Interim Report, Vol. II. : Pages 11 and 744 to 751 ; *Chart of Culture Inoculations*, page 747.

Final Report : Pages 9 to 13.

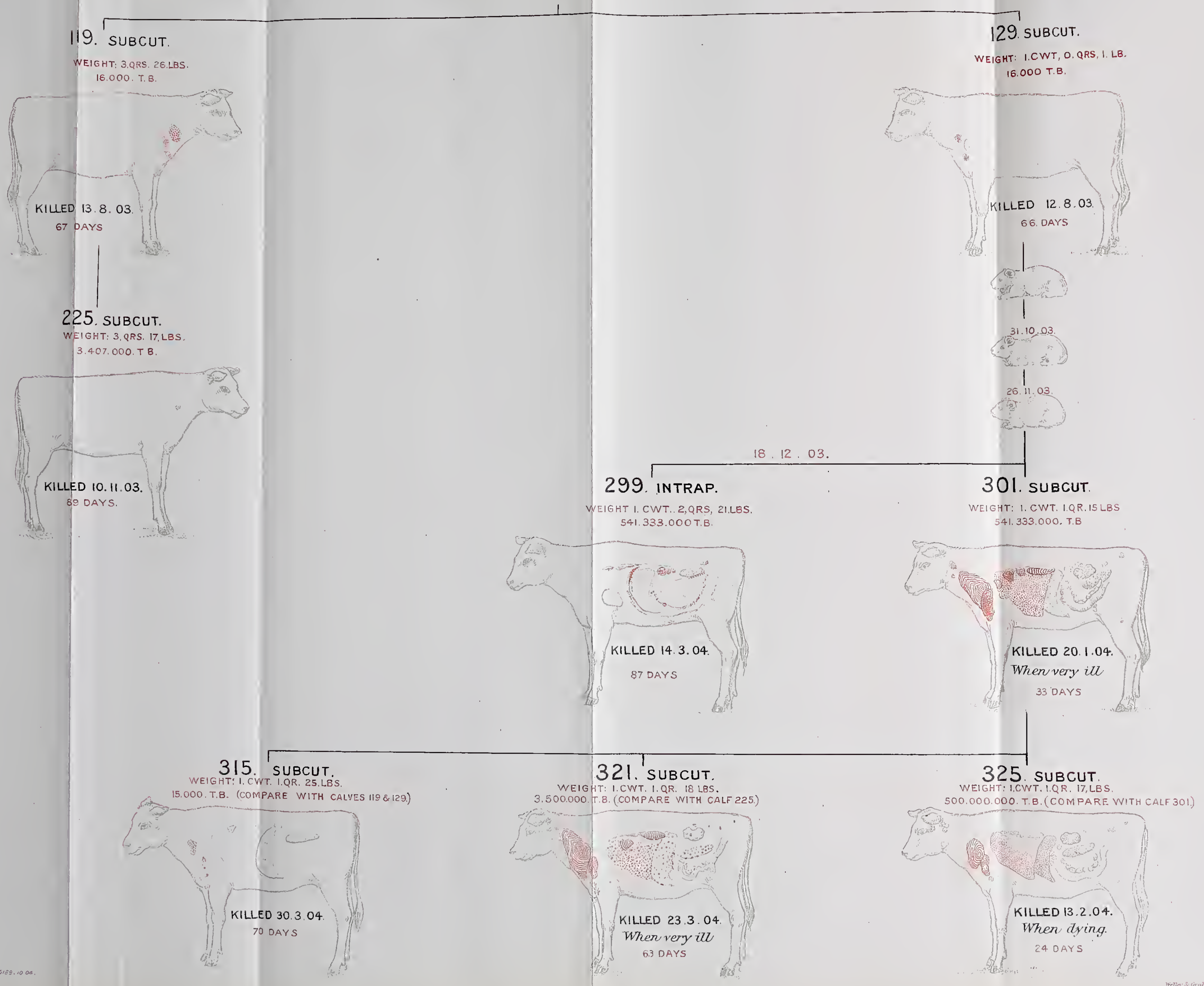
2.

10 Charts illustrating five viruses provisionally classed in Group III. in the 2nd Interim Report: one chart illustrating the further investigation of two of these viruses, and one chart illustrating two viruses subsequently investigated which presented similar anomalies.

Pages 30 to 42

BRONCHIAL GLANDS AND SPLEEN. VIRUS. H.13. "A.D."

7TH JUNE 1903.



5189.10.04.

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VIRUS H. 13. "A.D." Bronchial Glands and Spleen. June 7, 1903.

A virus obtained from a case of tuberculosis in man, from animals inoculated with which **both the human and the bovine tubercle bacillus were isolated.**

Nature of case : *General Tuberculosis* (primary seat of disease not ascertained).

Age of patient : 4 years.

Material used (*post-mortem*) : Bronchial Glands and Spleen (mixed emulsion) only.

Explanation of Charts.—The early experiments with this virus were made for the purpose of determining the virulence of the *emulsion* obtained from the original material.

After passage through the bodies of guinea-pigs, inoculated to increase the numbers of tubercle bacilli, the virulence of the virus appeared to be higher for the calf than it was originally. (In this series of experiments (Chart 25) *emulsions* of tuberculous lesions alone were employed.)

The investigation of the virus was therefore continued. Inoculation experiments were made (Chart 25A) on calves with two strains of *culture* obtained at successive stages of the "Passage" experiment (from Calf 301 and Calf 321.)

Charts 25 and 25A present the results which had been obtained with virus H. 13 "A.D." at the time of the issue of the 2nd Interim Report, results which caused the Commission to include it with those viruses placed provisionally in Group III.

References.—2nd Interim Report : Page 32, para. 55 ; page 33, para. 58 ; page 34, paras. 60 to 65 ; page 92.

Appendix to 2nd Interim Report, Vol. II. : Pages 6 and 279 to 302.

Further investigations were subsequently carried out with the strain of *culture* derived from Calf 301, which had been shown "to possess the capacity to grow luxuriantly on artificial media, and to produce general progressive tuberculosis in calves and rabbits."

These investigations demonstrated that "this want of that accordance which usually obtains between cultural characters and virulence was due to a mixture of bacilli, with the properties of the human and bovine tubercle bacillus respectively." (*See* Chart 30.)

References.—Final Report : Page 15.

Appendix to Final Report, Vol. III. : "Investigation of Certain Human Viruses of Irregular Type."

VIRUS H.13. "A.D."

CULTURE INOCULATIONS.

(Two strains of culture obtained at successive stages of the "Passage Experiment")

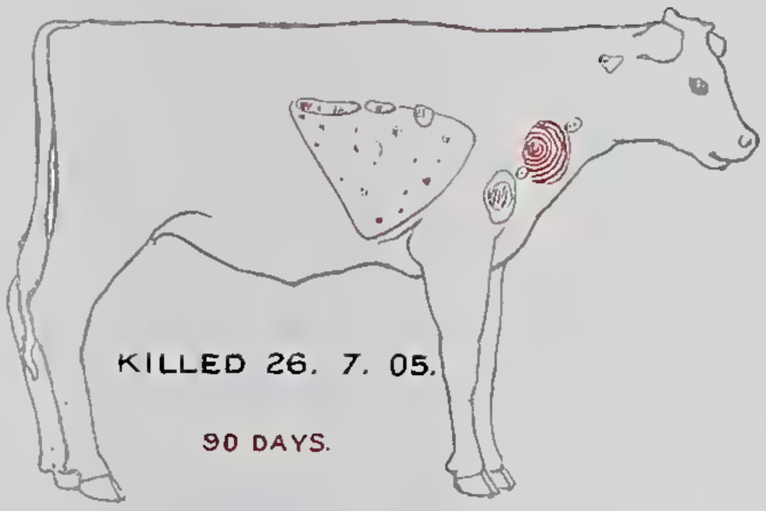
[1ST. PASSAGE.]

APRIL 27TH 1905.

Strain derived from Calf 301, direct.
Total duration of artificial cultivation, 15 months.

761. SUBCUT.

DOSE, 50 MILLIGRAMMES.

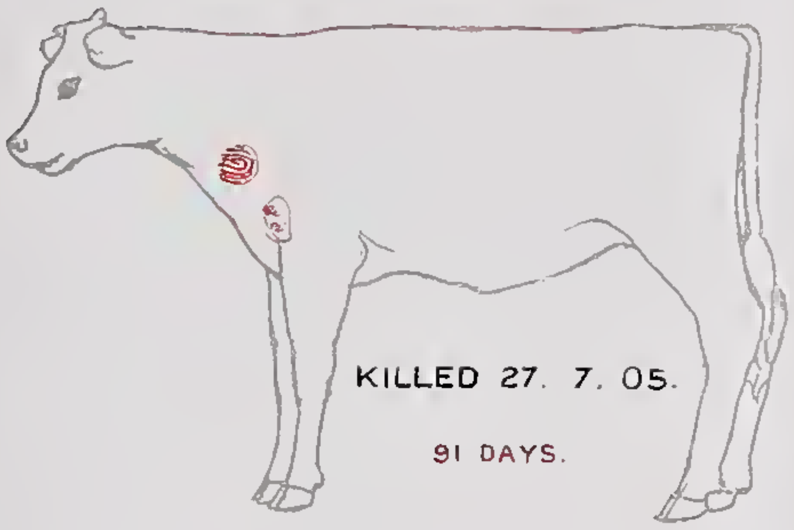


KILLED 26. 7. 05.

90 DAYS.

765. SUBCUT.

DOSE, 50 MILLIGRAMMES.



KILLED 27. 7. 05.

91 DAYS.

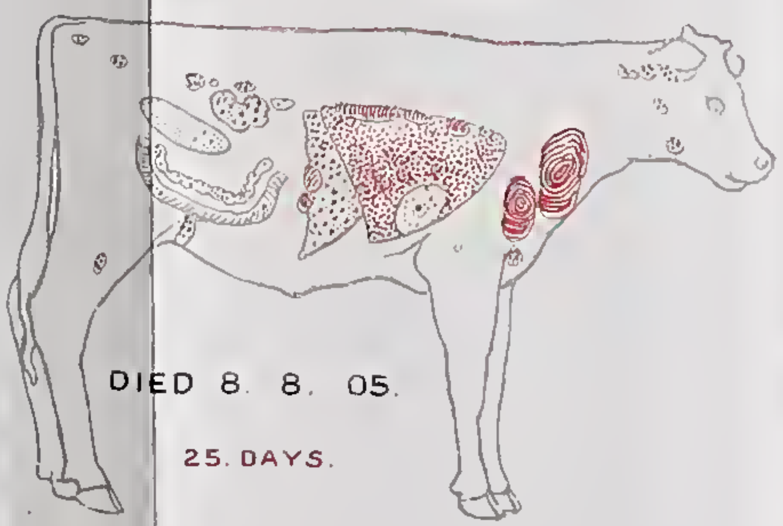
[2ND. PASSAGE.]

JULY 14TH 1905.

Strain derived from Calf 321, direct.
Total duration of artificial cultivation, 15½ months

825. SUBCUT.

DOSE, 50 MILLIGRAMMES.



DIED 8. 8. 05.

25. DAYS.

5189.

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CHART 25A.

VIRUS H. 13. "A.D." Culture Inoculations.

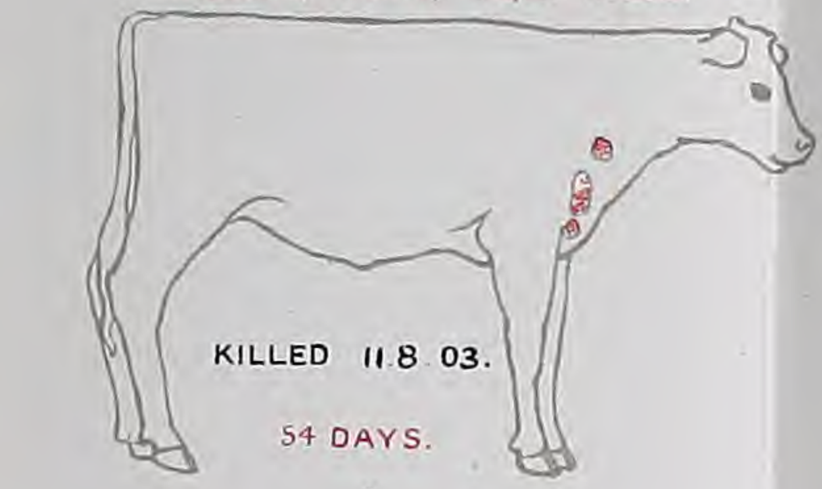
(*See* Chart 25.)

KNEEJOINT. VIRUS H.16. "J.H."

18TH JUNE, 1903.

155. SUBCUT.

DOSE, 10.000. T.B.
WEIGHT, 1 CWT, 1 QR. 11. LBS.

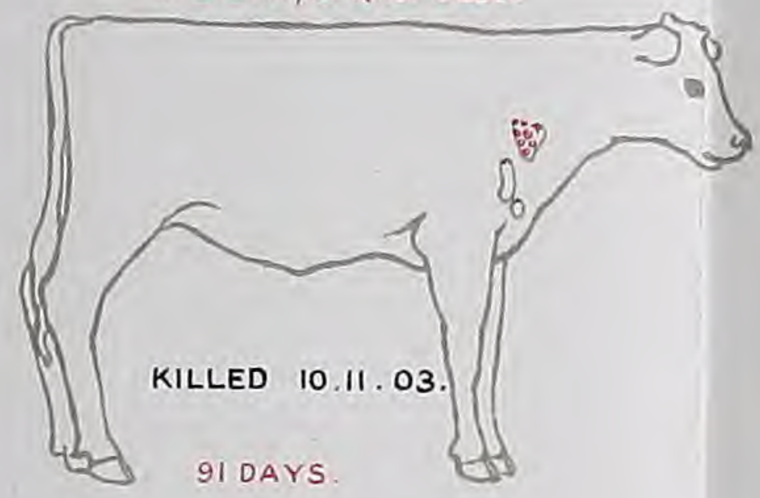


KILLED 11.8 03.

54 DAYS.

229. SUBCUT.

DOSE, 712.800. T.B.
WEIGHT, 3 QRS. 9 LBS.

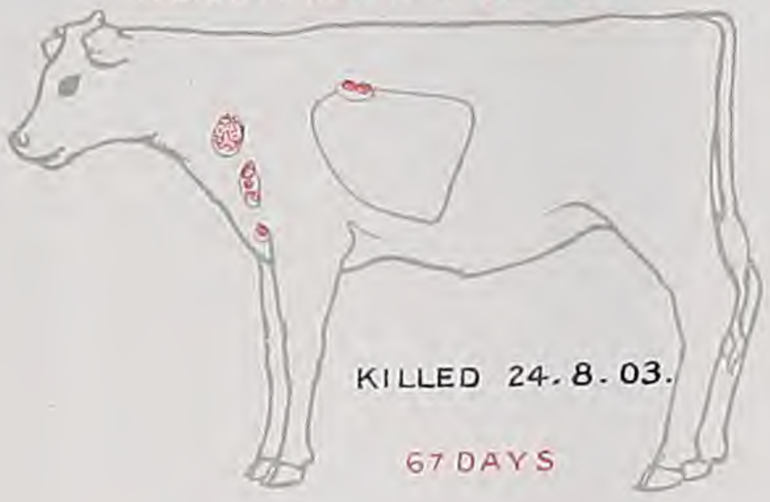


KILLED 10.11.03.

91 DAYS.

157. SUBCUT.

DOSE, 10.000 T.B.
WEIGHT, 1.CWT. 0.QR. 3.LBS

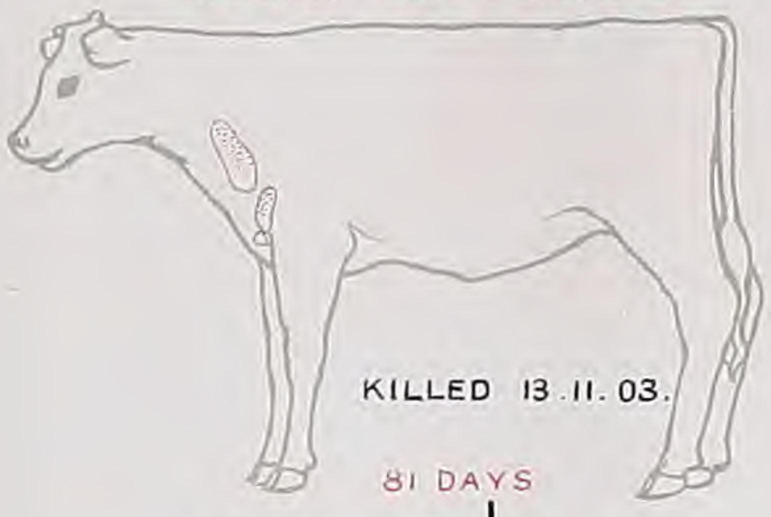


KILLED 24.8.03.

67 DAYS

187. SUBCUT.

DOSE, 3.003.000. T.B.
WEIGHT, 1 CWT. 2 QRS, 14 LBS.

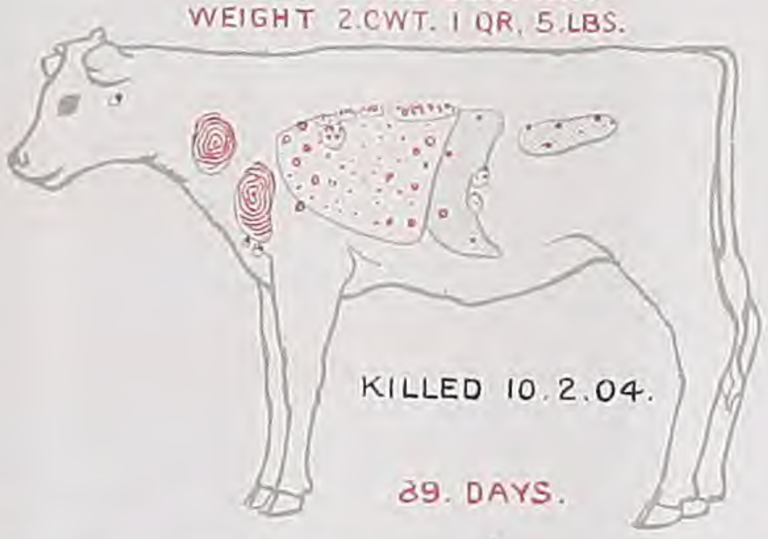


KILLED 13.11.03.

81 DAYS

273. SUBCUT.

DOSE, 10.903.000. T.B.
WEIGHT 2.CWT. 1 QR. 5.LBS.

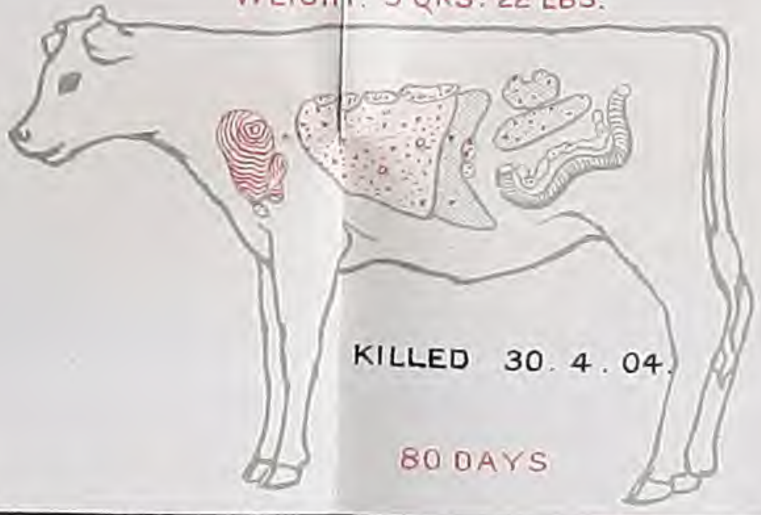


KILLED 10.2.04.

89. DAYS.

337. SUBCUT.

DOSE, 3.063.000. T.B.
WEIGHT. 3 QRS. 22 LBS.

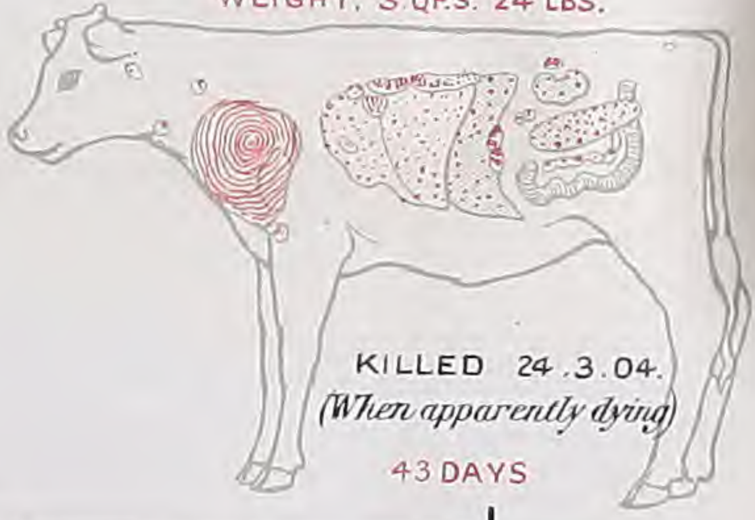


KILLED 30.4.04.

80 DAYS

355. SUBCUT.

DOSE, 3.063.000. T.B.
WEIGHT. 3 QRS. 24 LBS.

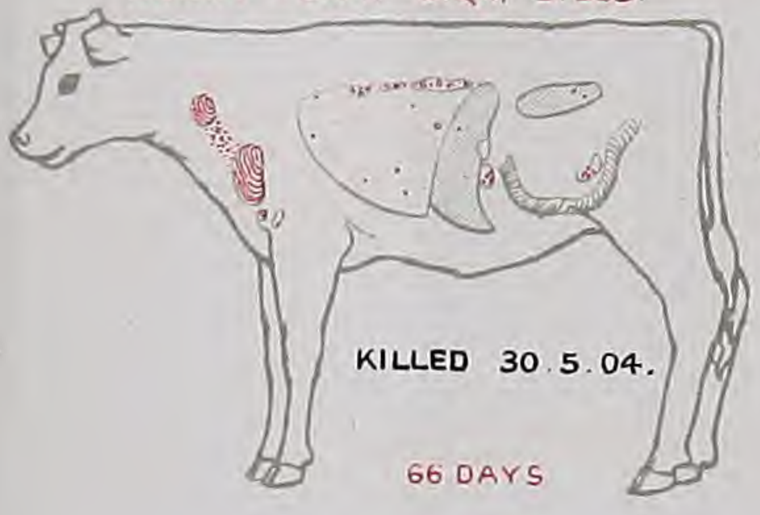


KILLED 24.3.04.
(When apparently dying)

43 DAYS

371. SUBCUT.

DOSE, 10.000.T.B. (COMPARE WITH CALVES 155 & 157.)
WEIGHT 1 CWT. 0.QR. 3.LBS.

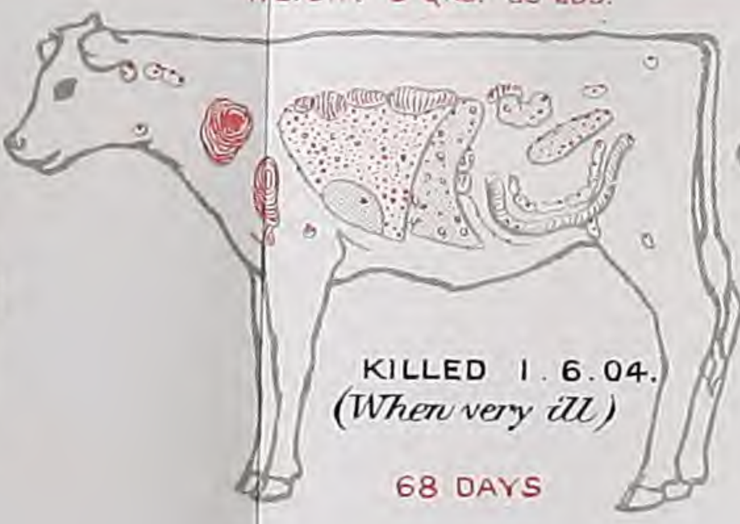


KILLED 30.5.04.

66 DAYS

343. SUBCUT.

DOSE, 712.000.T.B. (COMPARE WITH CALF 229)
WEIGHT 3 QRS. 23 LBS.

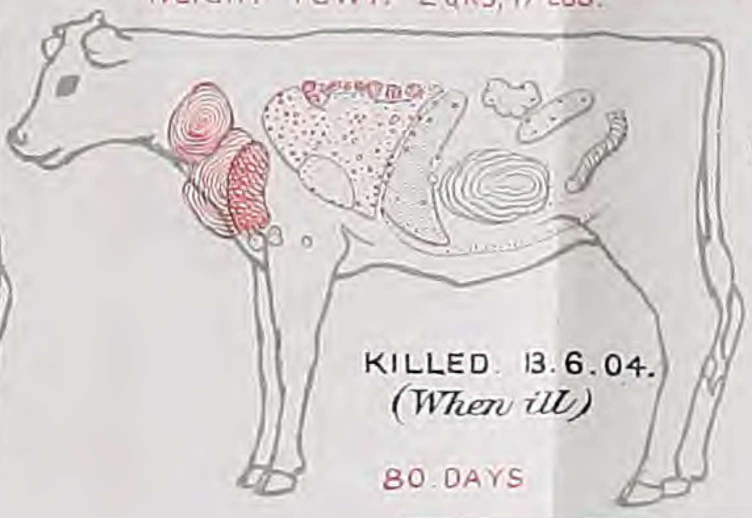


KILLED 1.6.04.
(When very ill)

68 DAYS

317. SUBCUT.

DOSE, 3.060.000.T.B. (COMPARE WITH CALVES 187, 337, & 355)
WEIGHT 1 CWT. 2 QRS, 17 LBS.

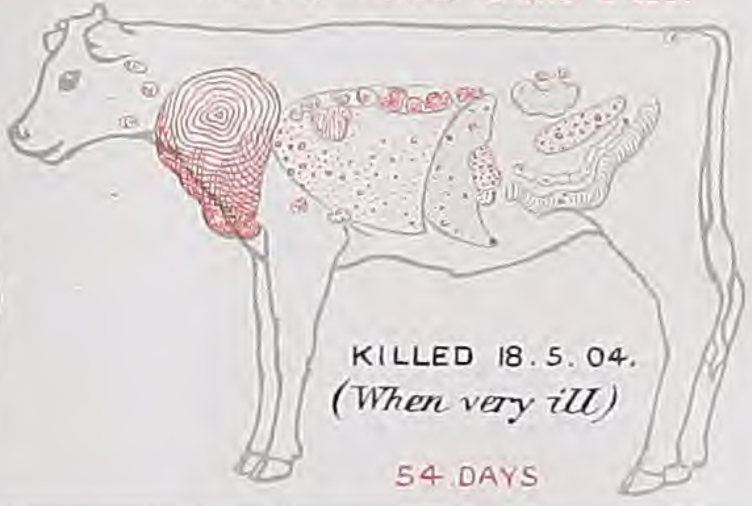


KILLED 13.6.04.
(When ill)

80 DAYS

281. SUBCUT.

DOSE, 10.900.000.T.B. (COMPARE WITH CALF 273)
WEIGHT 1 CWT. 3 QRS. 6 LBS.



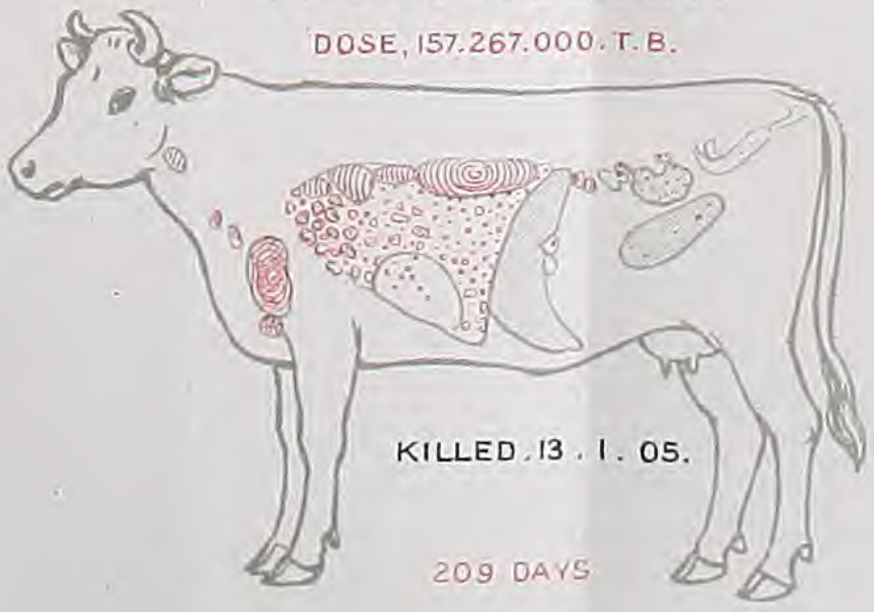
KILLED 18.5.04.
(When very ill)

54 DAYS

18.6.04.

245. SUBCUT.

DOSE, 157.267.000. T.B.

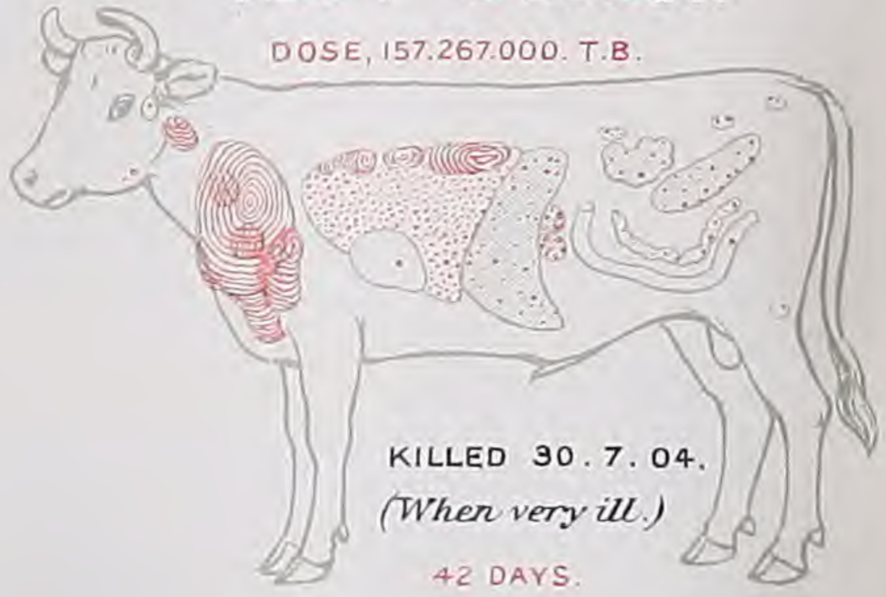


KILLED 13.1.05.

209 DAYS

423A. SUBCUT.

DOSE, 157.267.000. T.B.



KILLED 30.7.04.
(When very ill.)

42 DAYS.

5189

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CHARTS 26 and 26A.

VIRUS H. 16. "J.H." Knee-joint. June 18, 1903.

A virus obtained from a case of tuberculosis in man from animals inoculated with which **both the human and the bovine tubercle bacillus were isolated.**

Nature of case : *Joint Tuberculosis.*

Age of patient : 38 years.

Material used (*clinical*) **:** Synovial membrane of knee-joint.

Explanation of Charts.—The early experiments with this virus were made for the purpose of determining the virulence of the *emulsion* of human tissue. After passage through the bodies of two calves the virulence of the virus appeared to be persistently higher than it was originally. (In this series of experiments *emulsions* of tuberculous lesions were alone employed—*see* Chart 26.) The investigation was therefore continued. Inoculation experiments were made on calves with strains of *culture* obtained at successive stages of the passage experiment (*see* Chart 26A). The "primary strain" culture and the "2nd passage" culture were eugonic and grew luxuriantly like the human tubercle bacillus ; the cultures from the 3rd, 4th, and 5th passages were dysgonic, growing like the bovine tubercle bacillus.

These anomalous results caused the Commission, in the 2nd Interim Report, to include Virus H. 16. "J.H." with those viruses placed provisionally in Group III.

References.—2nd Interim Report : Page 31, para. 54 ; page 33, para. 58 ; page 34, paras. 60 to 65 ; page 94.

Appendix to 2nd Interim Report, Vol. II. : Pages 14 and 349–396.

Further investigations carried out by Dr. Griffith on two of the Group III. viruses, H. 13. "A.D." and H. 49. "T.C.", which presented irregular features similar to those of H. 16. "J.H.", showed that these anomalies were due to a mixture of bovine and human tubercle bacilli.

It appears highly probable, therefore, that in the case of Virus 16. "J.H." bovine tubercle bacilli as well as human were present in the *emulsion* of the original material inoculated into Calves 155 and 157.

References.—Final Report : Page 15.

Appendix to Final Report, Vol. III. : "Investigation of Certain Human Viruses of Irregular Type."

KNEEJOINT. VIRUS H.16 "J.H."

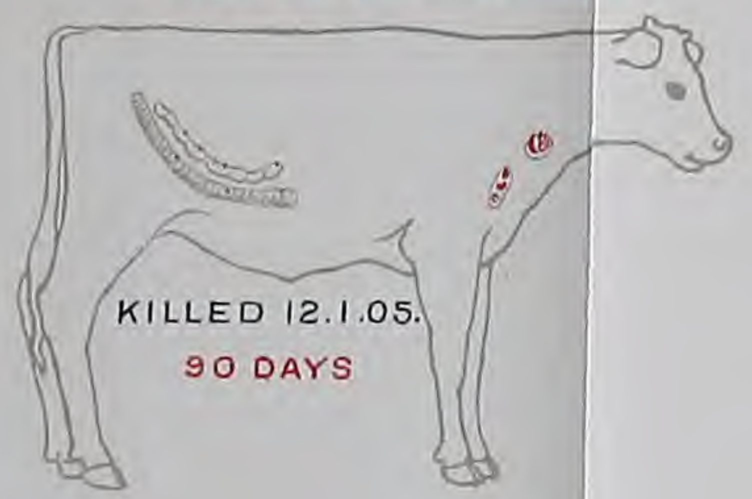
Calves inoculated with strains of
Culture obtained at various stages
of the subcutaneous passage experiment

PRIMARY STRAIN

Strain derived from Human synovial membrane, through G.P. 609.
Tested for Virulence after 14 months artifical cultivation.
Subculture on Pig Serum used, 22 days old.
14. 10. 04.

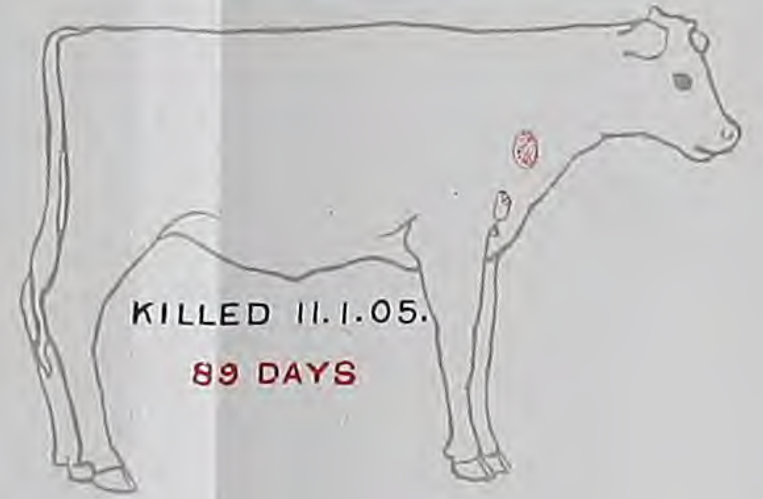
527. SUBCUT.

DOSE, 50 MILLIGRAMMES.



549. SUBCUT.

DOSE, 50 MILLIGRAMMES.

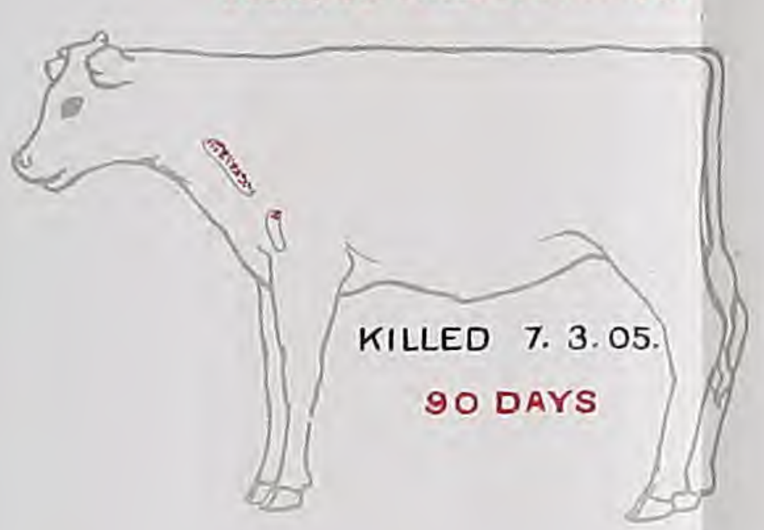


2ND. "PASSAGE"

Strain derived from Calf 273., direct.
Tested for Virulence after 10 months artificial cultivation.
Subculture on Pig Serum used, 22 days old.
7. 12. 04.

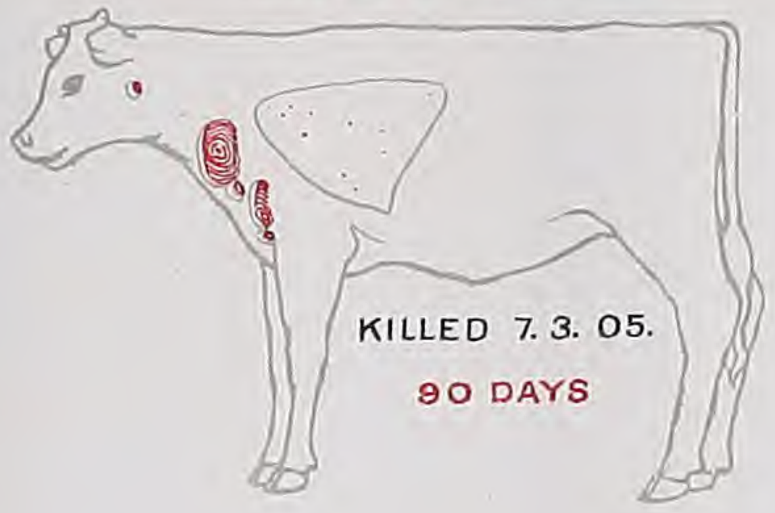
567. SUBCUT.

DOSE, 50 MILLIGRAMMES.



573. SUBCUT.

DOSE, 50 MILLIGRAMMES.

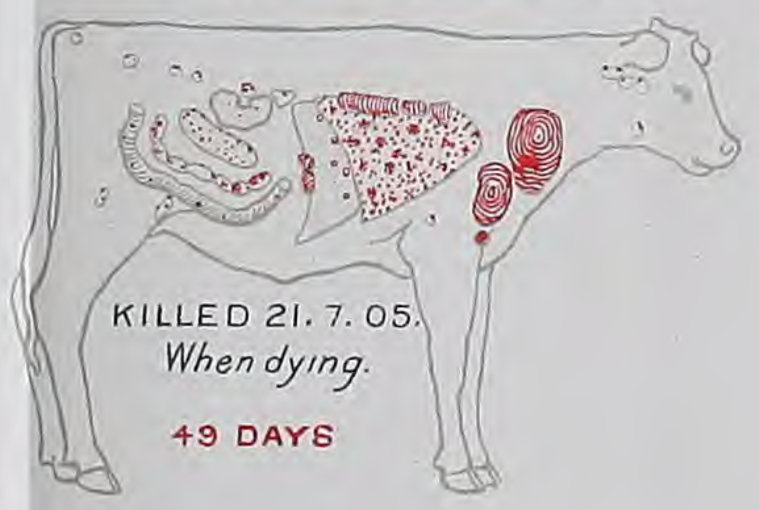


3RD. "PASSAGE"

Strain derived from Calf 337, direct.
Tested for Virulence after 13 months artificial cultivation
Subculture on bovine serum used, 21 days old.
2. 6. 05.

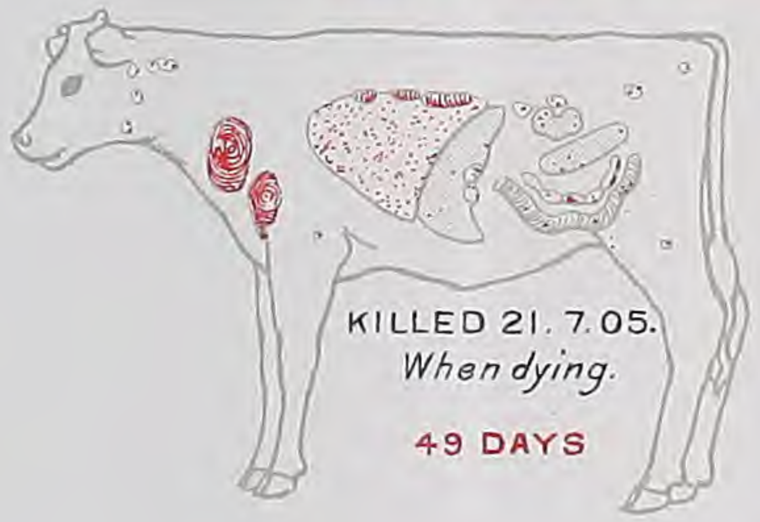
789. SUBCUT.

DOSE, 50 MILLIGRAMES.



795. SUBCUT.

DOSE, 50 MILLIGRAMMES.

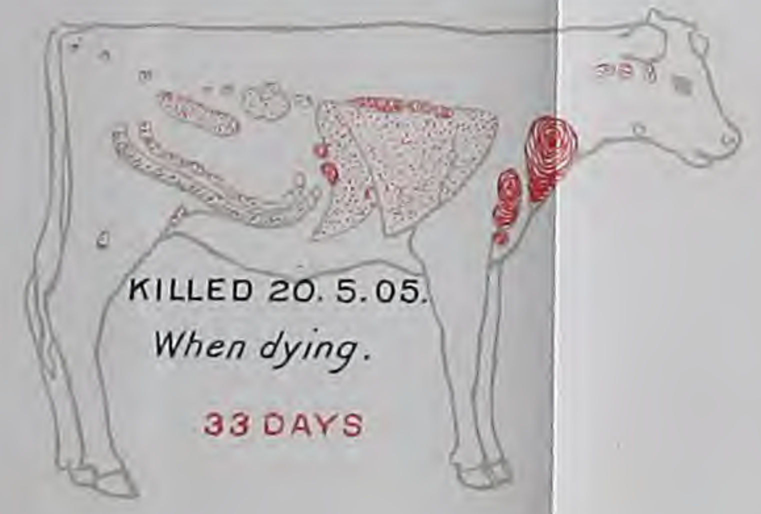


4TH "PASSAGE."

Strain derived from Calf 317, direct.
Tested for Virulence after 10 months artificial cultivation.
Subculture on bovine serum used, 21 days old.
17. 4. 05.

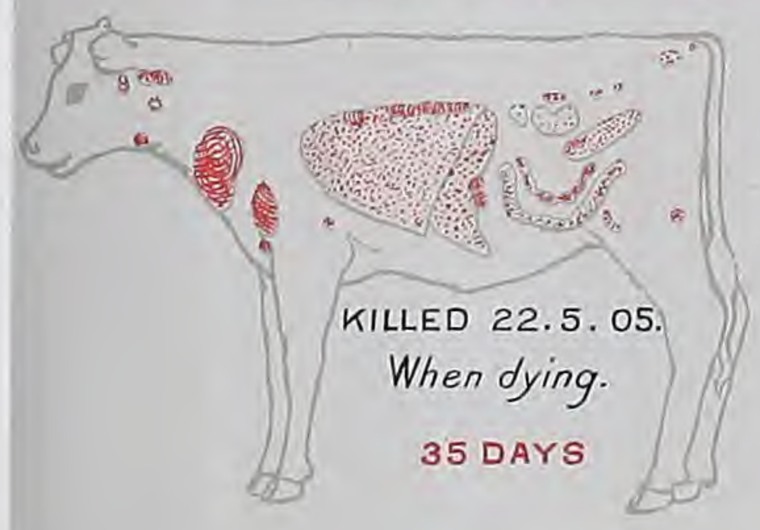
711. SUBCUT.

DOSE, 50 MILLIGRAMMES.



729. SUBCUT.

DOSE, 50 MILLIGRAMMES.

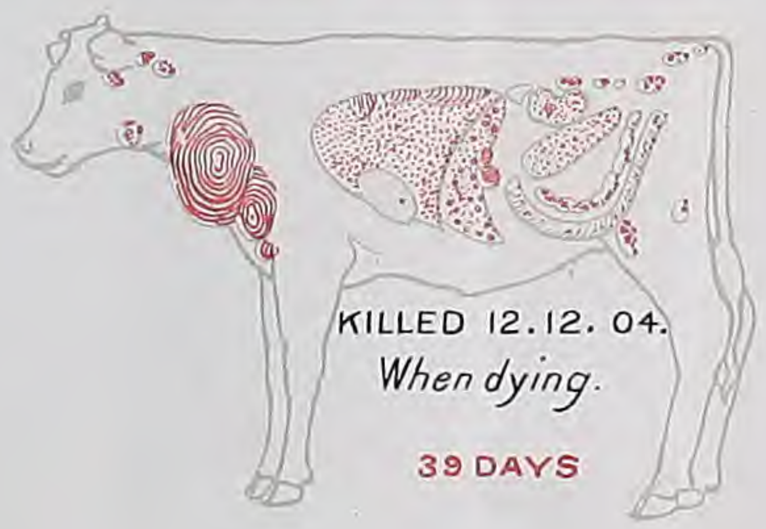


5TH "PASSAGE."

Strain derived from Bull 423A direct.
Tested for Virulence after 3 months artificial cultivation
Subculture on Pig Serum used, 21 days old.
3. 11. 04.

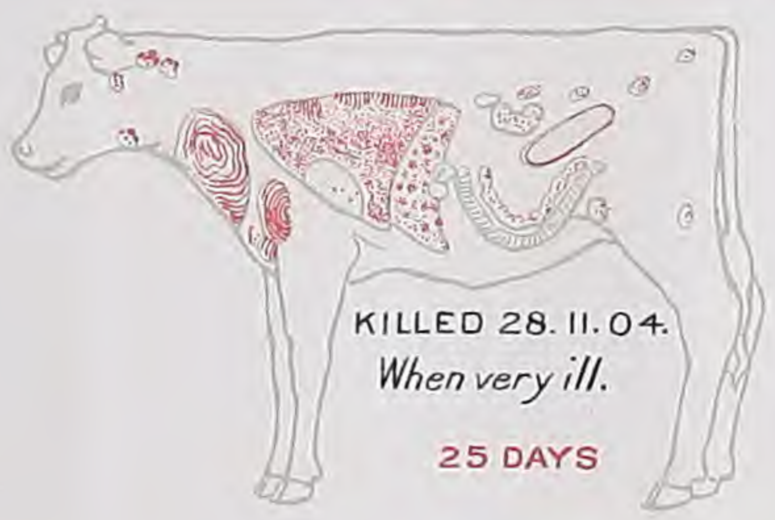
559. SUBCUT.

DOSE, 50 MILLIGRAMMES.



541. SUBCUT.

DOSE, 50 MILLIGRAMMES.



5189.

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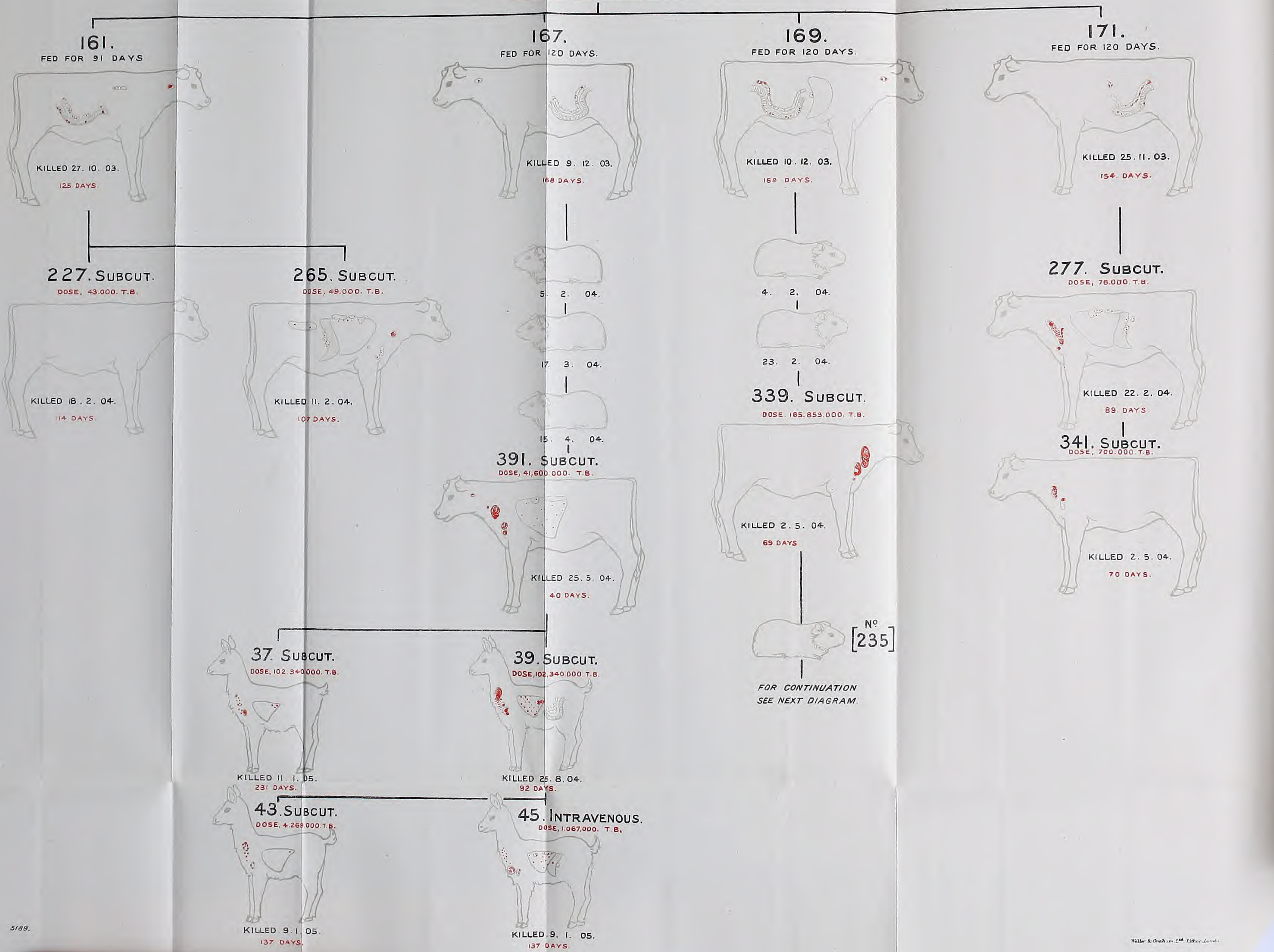
CHART 26A.

VIRUS H. 16. "J.H." Culture Inoculations.

(*See* Chart 26.)

SPUTUM. VIRUS H.17. SP. B.

24TH JUNE, 1903.



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CHARTS 27, 27A, and 27B.

VIRUS H. 17. "Sp. B." Sputum. June 24, 1903.

The original material of the virus was the mixed sputum of many individuals suffering from pulmonary tuberculosis, which was subsequently **found to contain bovine as well as human tubercle bacilli.**

Nature of Disease: *Phthisis.*

Material used (*clinical*) : Mixed sputum from many patients.

Explanation of Charts.—The early experiments with this virus were made for the purpose of determining virulence. Calves were *fed with the sputum*, and passage experiments with *emulsion* of tuberculosis material derived from these animals were carried out on calves and goats. (*See* Chart 27.)

During the progress of the certain "intravenous passage experiment" (Charts 27 and 27A) an apparent increase in virulence of the virus took place. Strains of *culture* obtained at successive stages of this passage experiment were therefore tested as to their virulence for calves. (*See* Chart 27B.)

The three charts in question present the results which had been obtained with Virus H. 17. "Sp. B." at the time of the issue of the 2nd Interim Report, and which caused the Commission to include it amongst those viruses placed provisionally in Group III.

References.—2nd Interim Report: Page 19, para. 35 ; page 32, para. 56 ; page 34, paras. 58 and 60 to 65 ; page 90.

Appendix to 2nd Interim Report, Vol. II. : Pages 4 and 397 to 452.

Further investigations carried out by Dr. Stanley Griffith on two of the Group III. viruses, H. 13. "A.D." and H. 49. "T.C.", which had presented features similar to those of Virus H. 17. "Sp. B.", showed that these irregularities were caused by a mixture of bovine and human tubercle bacilli.

It appears highly probable therefore that in the case of Virus H. 17. "Sp. B." bovine bacilli as well as human had been present in the sputum with which the calves were fed.

References.—Final Report: Pages 14 and 15.

Appendix to Final Report, Vol. III. : "Investigation of Certain Human Viruses of Irregular Type."

VIRUS H. 17. "SP B."

CONTINUED FROM PREVIOUS DIAGRAM.

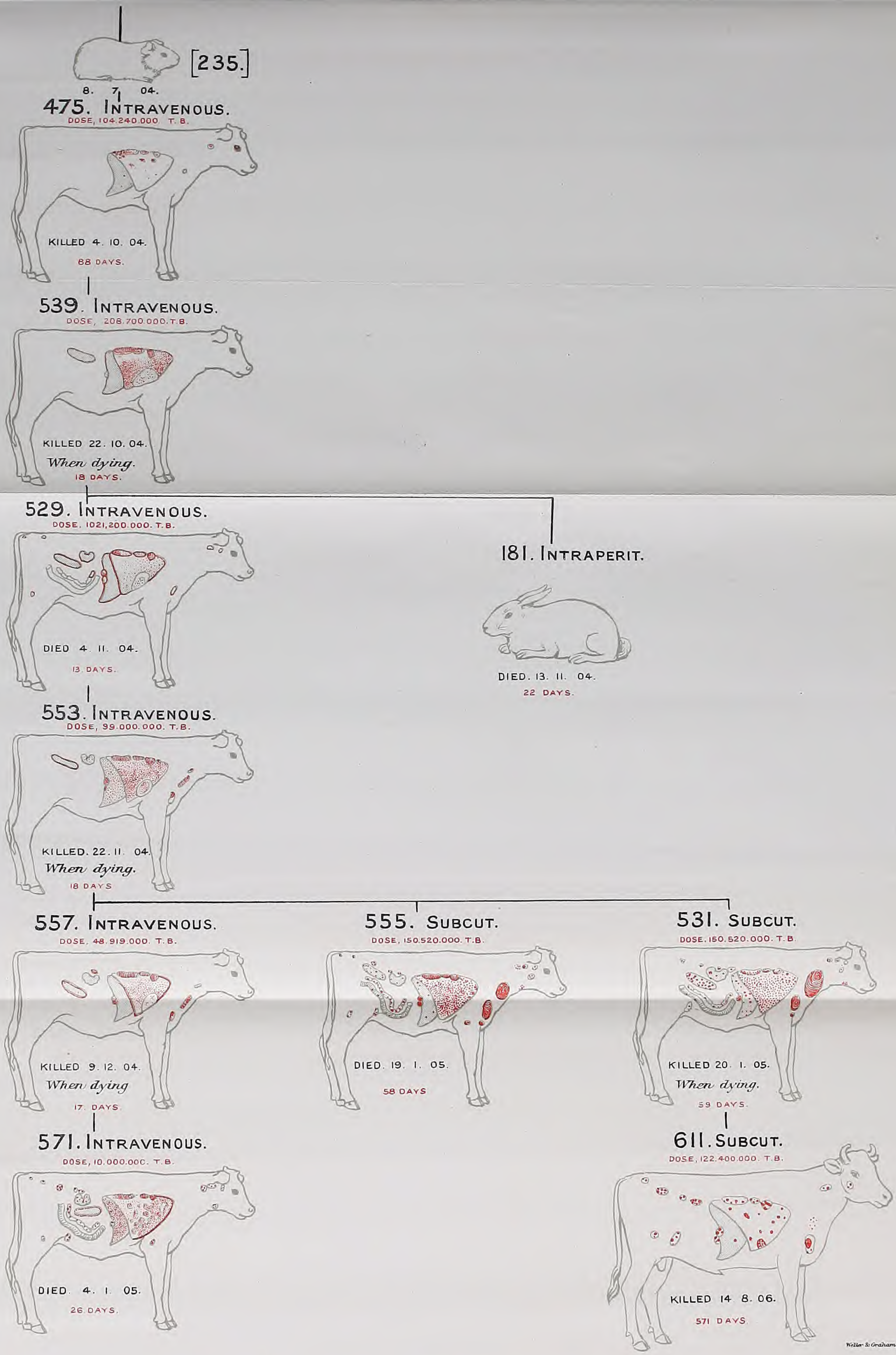

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CHART 27A.

Completion of the "Passage" Experiments with Virus H. 17. "Sp. B."

(*See* Chart 27.)

VIRUS H. 17. "SP. B."

CULTURE INOCULATIONS.

STRAINS OF CULTURE OBTAINED AT SUCCESSIVE STAGES OF THE "INTRAVENOUS PASSAGE EXPERIMENT"

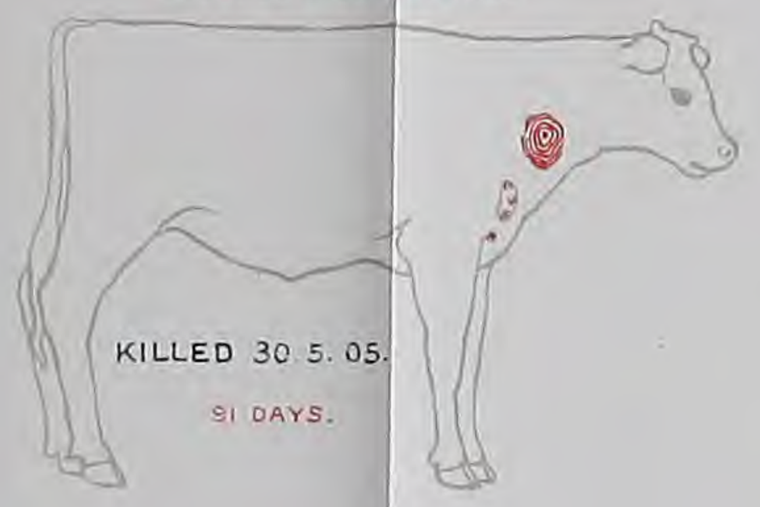
1ST. PASSAGE.

FEBRUARY 28TH 1905.

STRAIN DERIVED FROM CALF 339, THROUGH G.P. 1235.
TOTAL DURATION OF ARTIFICIAL CULTIVATION 8 MONTHS.

679. SUBCUT.

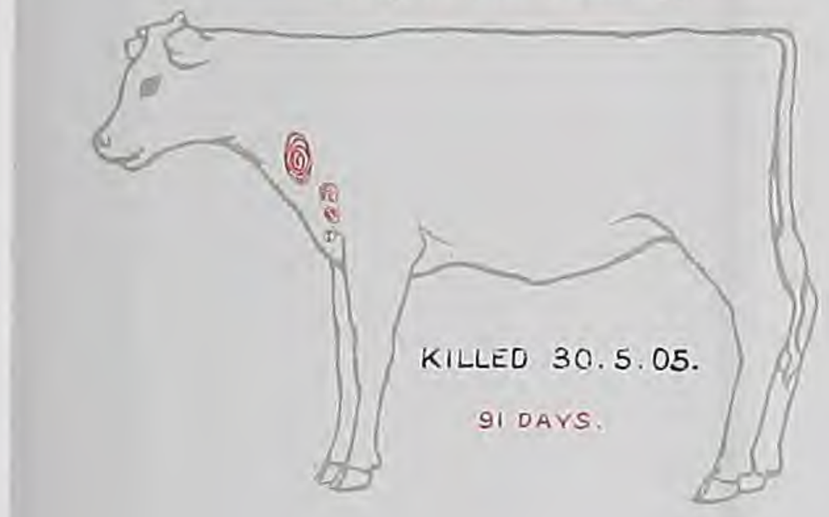
DOSE, 50 MILLIGRAMMES.



KILLED 30.5.05.
91 DAYS.

681. SUBCUT.

DOSE, 50 MILLIGRAMMES.



KILLED 30.5.05.
91 DAYS.

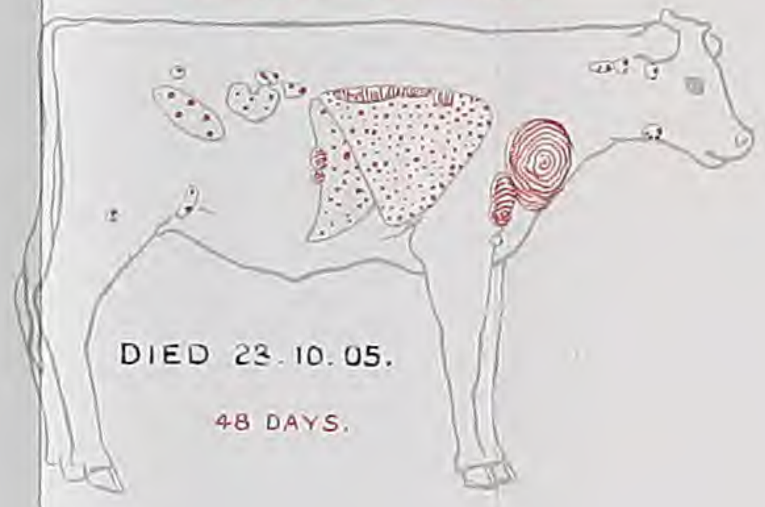
3RD PASSAGE.

SEPTEMBER 5TH 1905.

STRAIN DERIVED FROM CALF 539, THROUGH RABBIT 181.
TOTAL DURATION OF ARTIFICIAL CULTIVATION. 10 MONTHS.

891 SUBCUT.

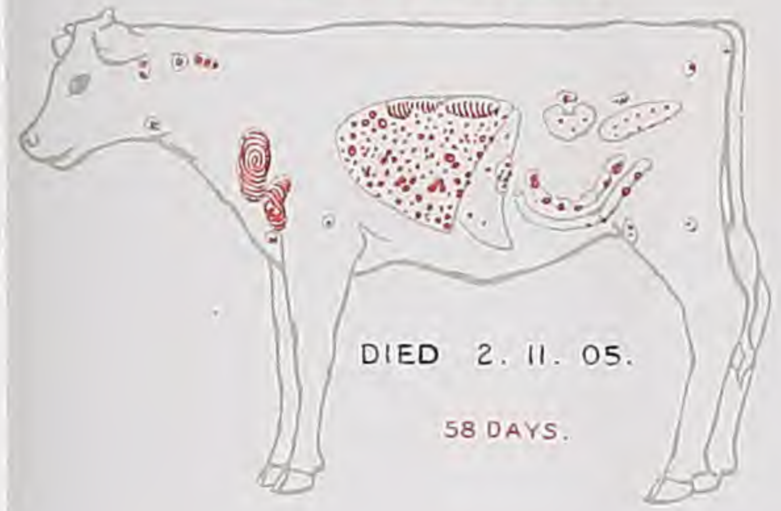
DOSE, 50 MILLIGRAMMES.



DIED 23.10.05.
48 DAYS.

911. SUBCUT.

DOSE, 50, MILLIGRAMMES.



DIED 2.11.05.
58 DAYS.

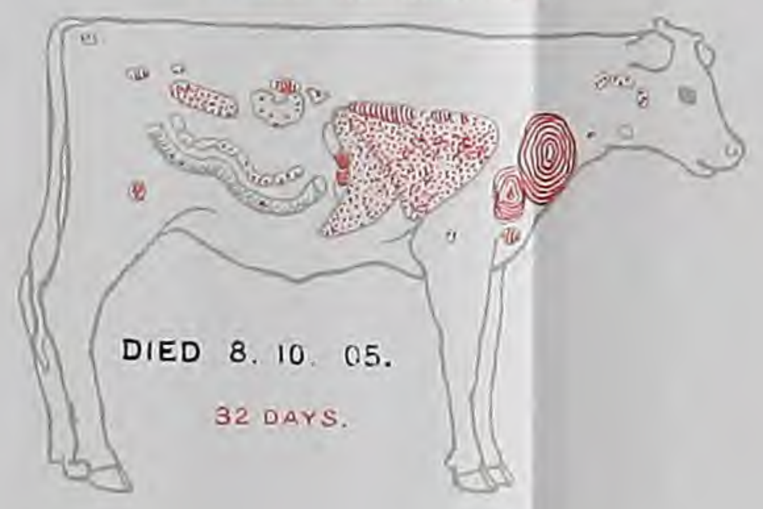
5TH PASSAGE.

SEPTEMBER 6TH 1905.

STRAIN DERIVED FROM CALF 553 THROUGH G.P. 1542.
TOTAL DURATION OF ARTIFICIAL CULTIVATION, 3 MONTHS

897. SUBCUT.

DOSE, 50 MILLIGRAMMES.



DIED 8.10.05.
32 DAYS.

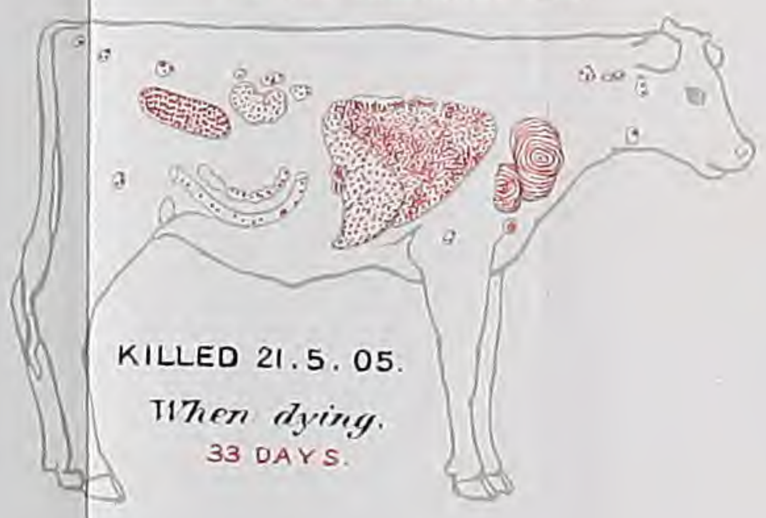
7TH PASSAGE.

APRIL 18TH 1905.

STRAIN DERIVED FROM CALF 571, DIRECT.
TOTAL DURATION OF ARTIFICIAL CULTIVATION, 3½ MONTHS.

739. SUBCUT.

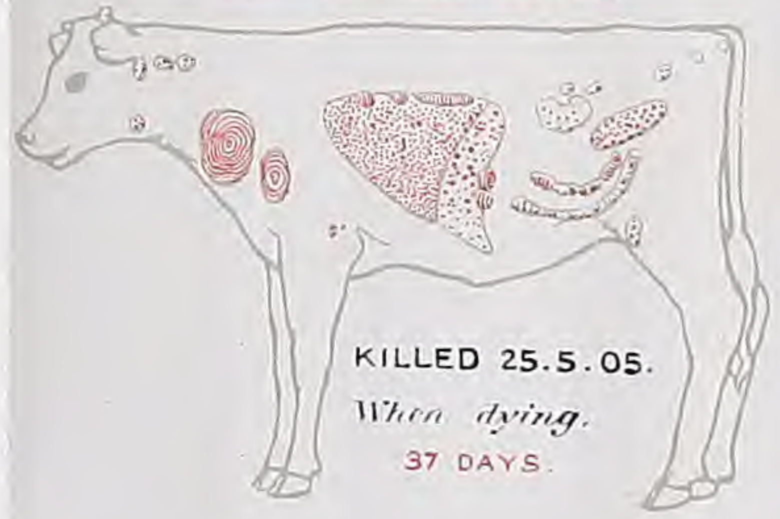
DOSE, 50. MILLIGRAMMES.



KILLED 21.5.05.
When dying.
33 DAYS.

743 SUBCUT.

DOSE. 50. MILLIGRAMMES.



KILLED 25.5.05.
When dying.
37 DAYS.

5/89.

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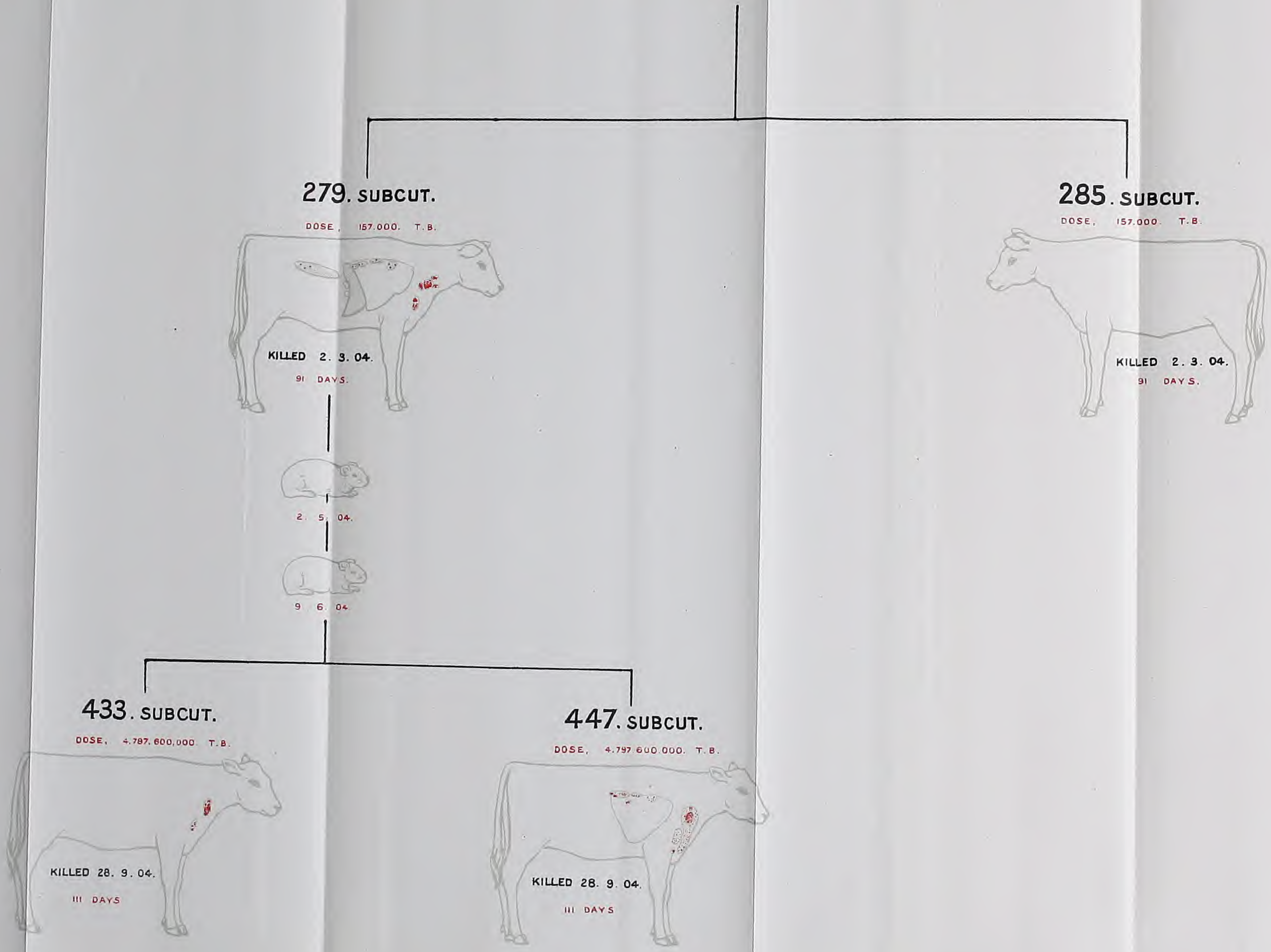
VIRUS H. 17. "Sp. B." Culture Inoculations.

(*See* Chart 27.)

BRONCHIAL GLANDS, VIRUS H. 21. "G.B."

2ND DECEMBER, 1903.



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CHARTS 28 and 28A.

VIRUS H. 21. "G.B." Bronchial Glands. December 2, 1903.

A virus obtained from a case of tuberculosis in man from a calf inoculated with which both the bovine and the human tubercle bacillus were isolated.

Nature of case: *Bronchial Gland Tuberculosis.*

Age of patient: 16 years.

Material used (*post-mortem*)**:** Bronchial Glands.

Explanation of Charts.—Inoculation experiments (Chart 28) on calves, with tissue *emulsions*, made (*a*) to determine the original virulence of the virus, and (*b*) the virulence after passage.

Later, *cultures* obtained from Calf 447 were tested on calves (*see* Chart 28A) and were found to be virulent for these animals.

The virus was excluded from discussion in the 2nd Interim Report (for reasons stated), and these charts are now included because the virus is dealt with in Dr. Cobbett's report, published in the Appendix to the Final Report.

References.—2nd Interim Report: Page 93.

Appendix to 2nd Interim Report, Vol. II.: Pages 6 and 515–530.

Appendix to Final Report, Vol. III.: "Report on the Stability of Tubercle Bacilli in the Living Animal."

VIRUS H.21. "G.B".

BRONCHIAL GLANDS.

CULTURE INOCULATIONS.

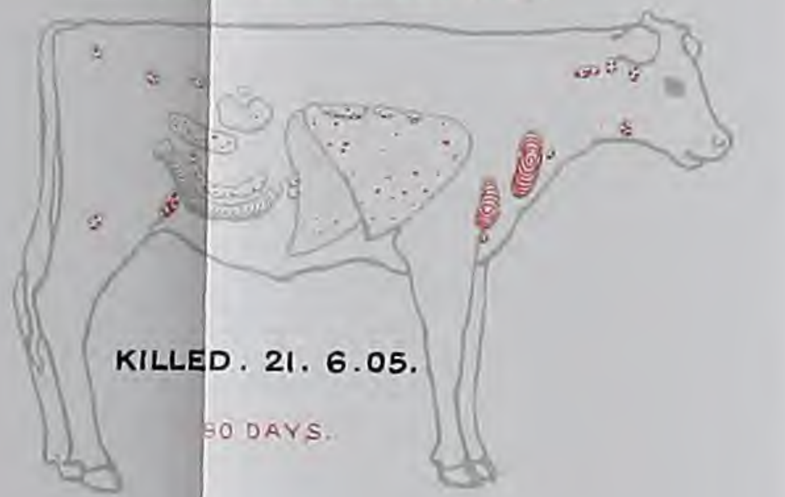
I

Strain derived from Guineapig 1417. (infected from the Prescapular Gland of Calf 447)
Tested for virulence after 4 Months artificial cultivation.
Third Subculture on bovine serum used, 21 days old.

23. 3. 05.

665. SUBCUT.

DOSE. 50 MILLIGRAMMES.

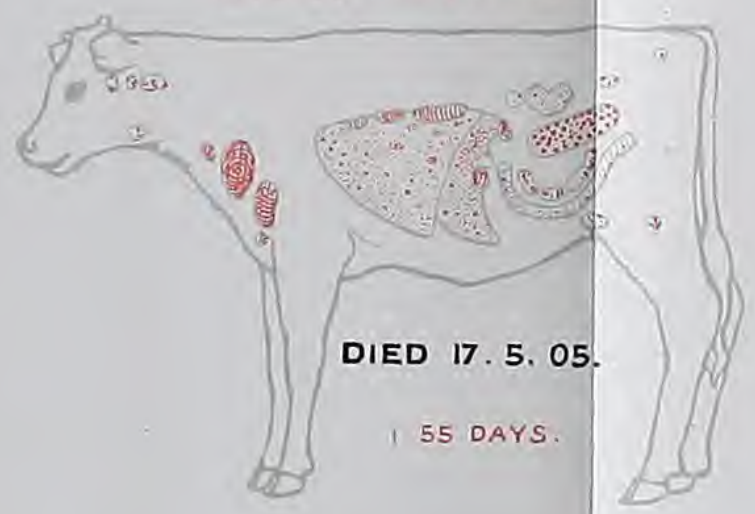


KILLED. 21. 6. 05.

0 DAYS.

669. SUBCUT.

DOSE. 50 MILLIGRAMMES.



DIED 17. 5. 05.

55 DAYS.

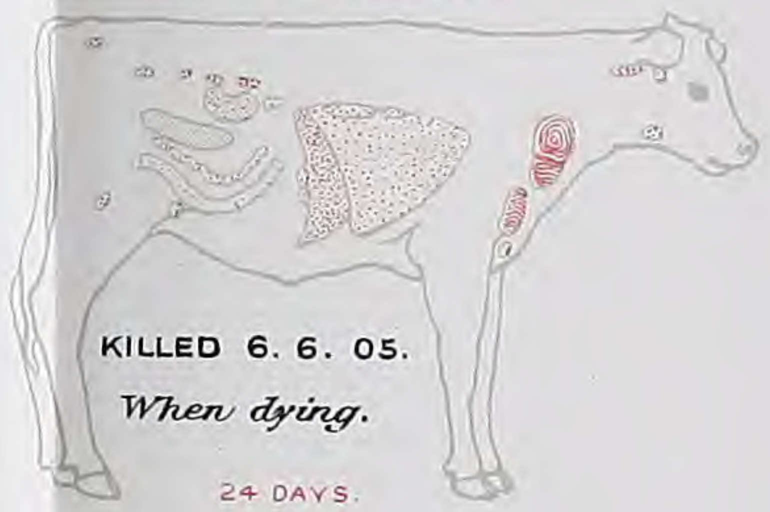
II

Strain derived directly from the thoracic gland of Calf 447.
Tested for virulence after 7½ Months artificial cultivation.
Sixth Subculture on bovine serum used, 28 days old.

13. 5. 05.

747. SUBCUT.

DOSE. 50 MILLIGRAMMES.



KILLED 6. 6. 05.
When dying.

24 DAYS.

5189

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CHART 28A.

VIRUS H. 21. "G.B." Culture Inoculations.

(*See* Chart 28.)

VIRUS H. 49 "T.C."

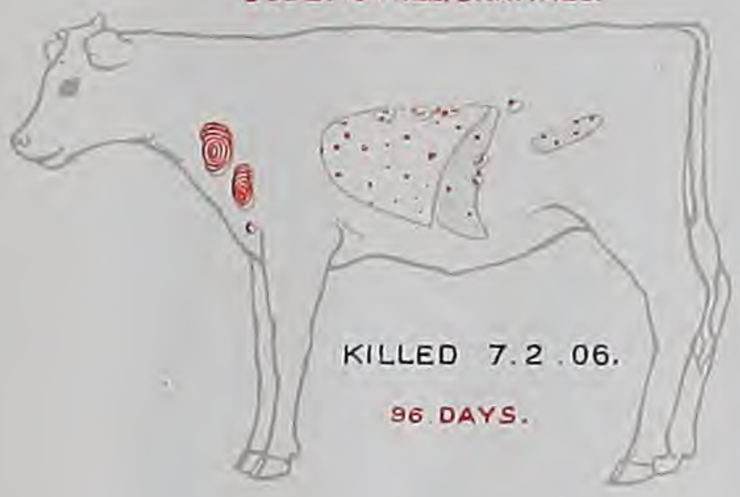
MESENTERIC GLANDS.

CULTURE INOCULATIONS.

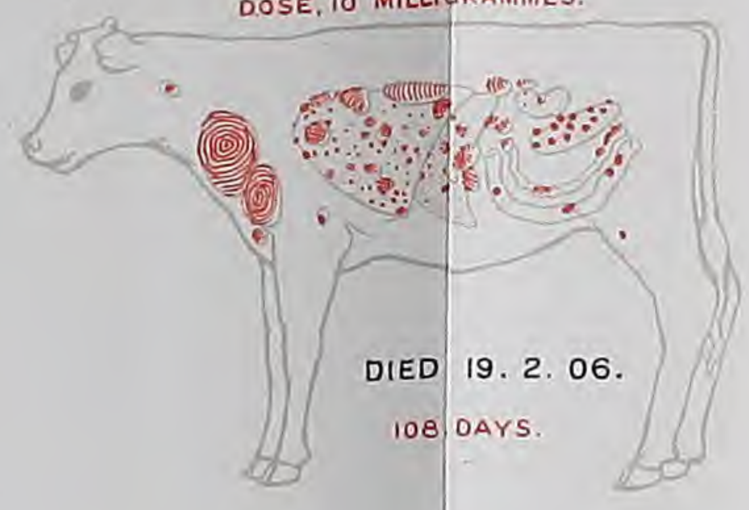
[II] NOVEMBER, 3RD 1905.

Strain derived from Human Mesenteric Glands, direct.
Total duration of artificial cultivation, 7 Months.

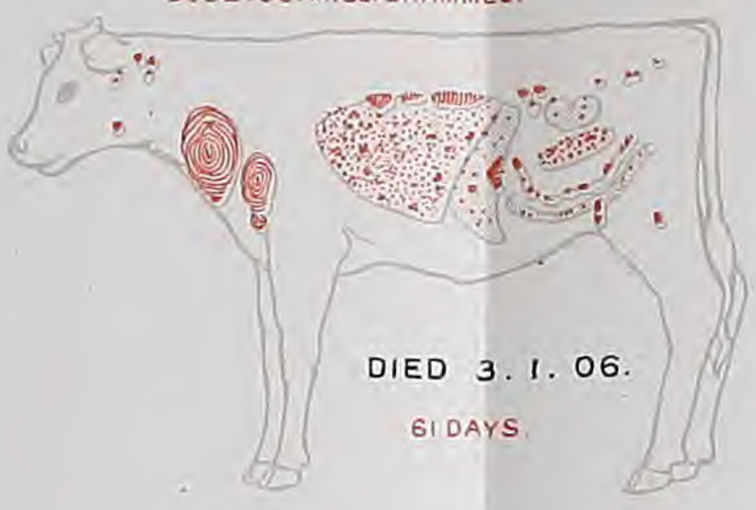
945. SUBCUT.
DOSE. 10 MILLIGRAMMES.



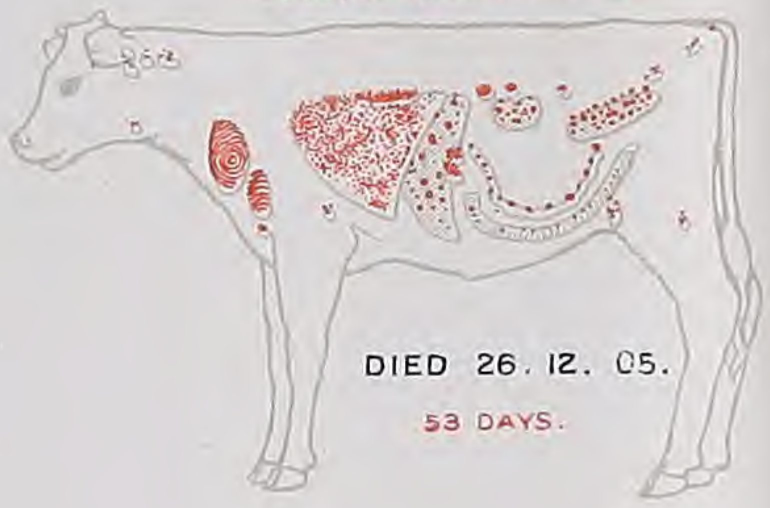
953. SUBCUT.
DOSE, 10 MILLIGRAMMES.



957. SUBCUT.
DOSE. 50 MILLIGRAMMES.



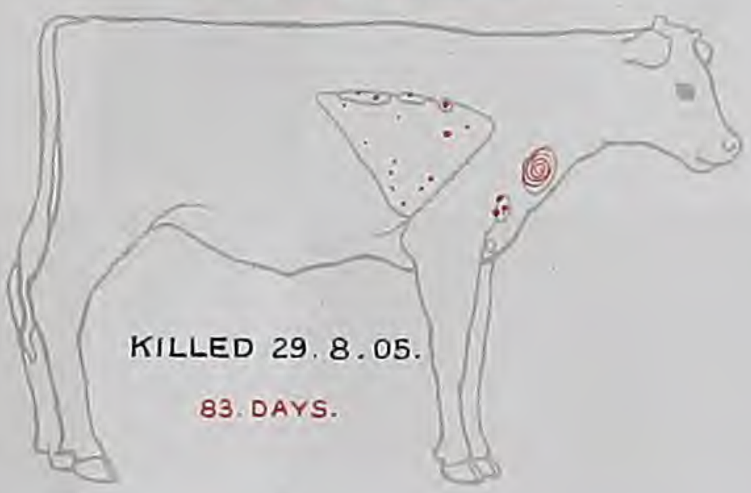
959. SUBCUT.
DOSE, 50 MILLIGRAMMES.



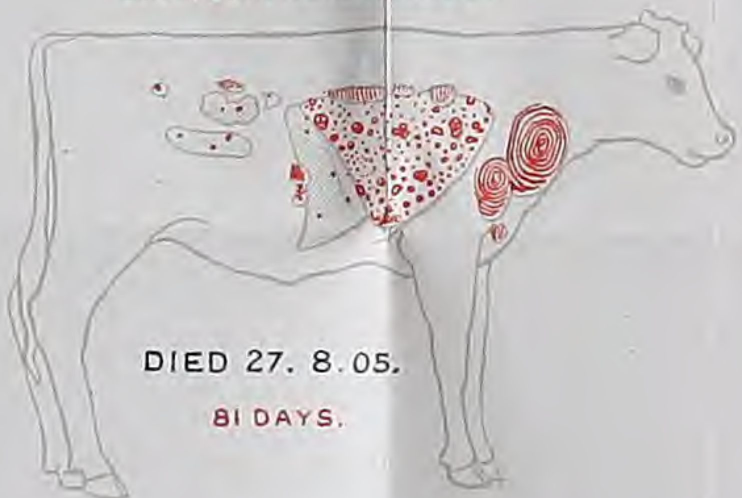
[I] JUNE, 7TH 1905.

Strain derived from Human Mesenteric Glands, direct.
Total duration of artificial cultivation, 2 Months.

797. SUBCUT.
DOSE. 50 MILLIGRAMMES.



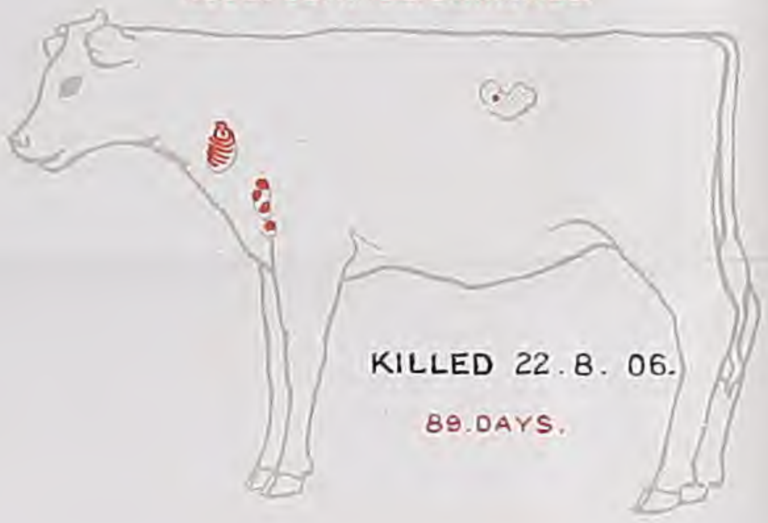
787. SUBCUT.
DOSE. 50. MILLIGRAMMES.



[III] MAY, 25TH 1906.

Strain derived from Human Mesenteric Glands, direct.
Total duration of artificial cultivation. 13½ Months.

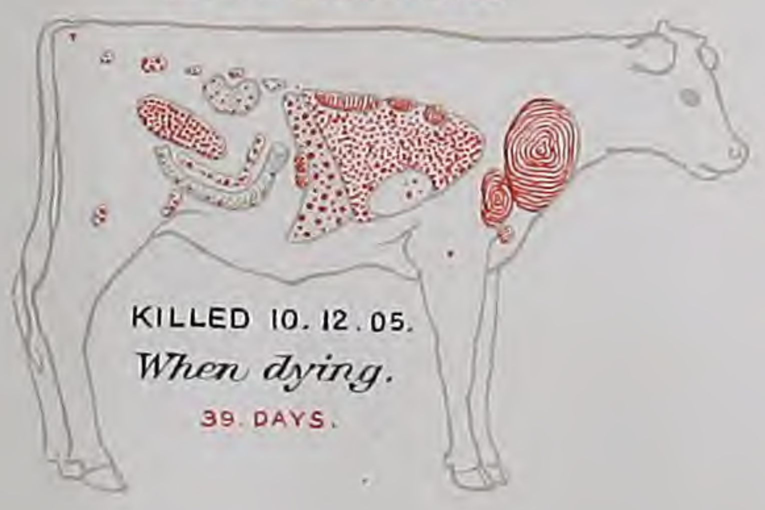
1097. SUBCUT.
DOSE. 50. MILLIGRAMMES.



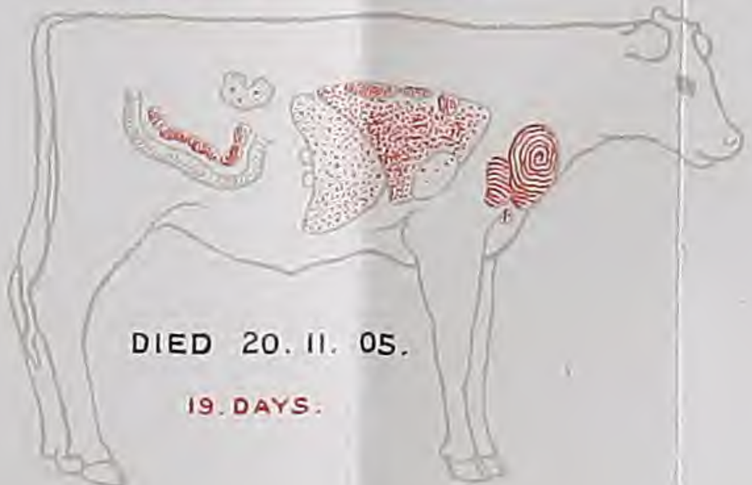
NOVEMBER, 1ST 1905.

Strain derived from Calf 797 direct.
Total duration of artificial cultivation, 2 Months.

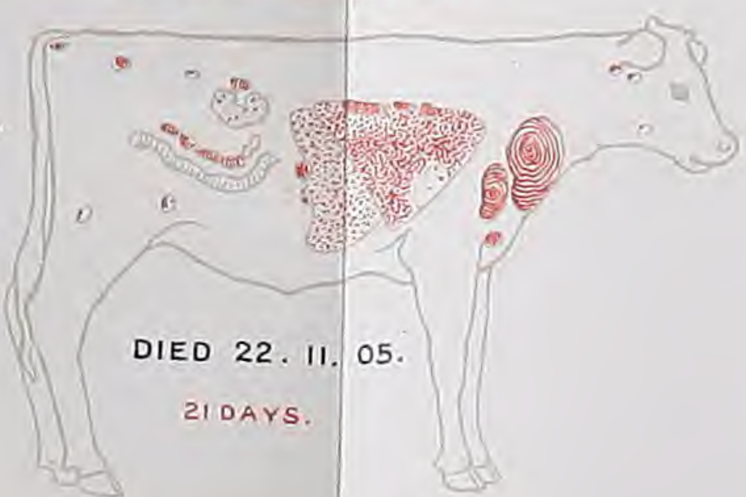
929. SUBCUT.
DOSE. 10 MILLIGRAMMES.



933. SUBCUT.
DOSE, 10 MILLIGRAMMES.



931. SUBCUT.
DOSE. 50 MILLIGRAMMES.



949. SUBCUT.
DOSE. 50. MILLIGRAMMES.

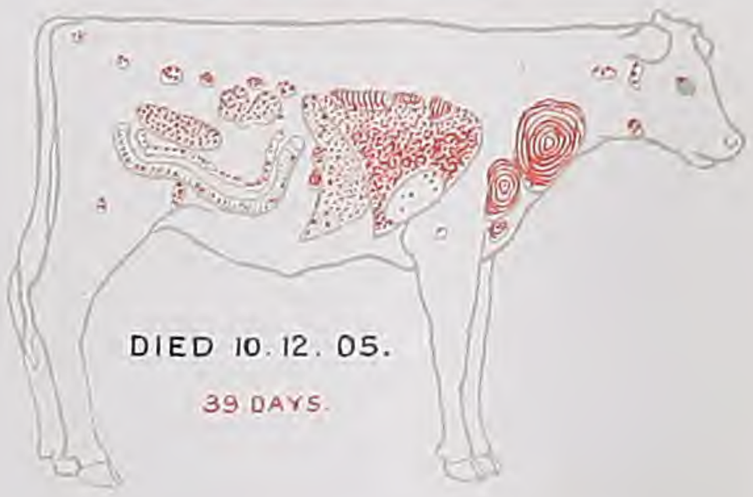


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CHART 29.

VIRUS H. 49. "T.C." Mesenteric Glands. April 6, 1905.

A virus obtained from a case of tuberculosis in man, subsequently shown to have been **a mixed infection of bovine and human tubercle bacilli.**

Nature of case: *Primary Abdominal Tuberculosis.*
Age of patient: 18 years.
Material used (*post-mortem*): Mesenteric Glands.

Explanation of Chart.—Inoculation experiments (Chart 29) on calves were made to determine the virulence of the *culture* obtained from the original material, and to test the virulence of the virus after passage through the body of the calf.

It was found that the original virulence fell a little below that of a bovine tubercle bacillus, but that after passage through the calf H. 49. "T.C.," was fully equal to a bovine virus in its virulence for the calf.

These irregular results caused the Commission, in the 2nd Interim Report, to include Virus H. 49. "T.C." amongst those viruses provisionally placed in Group III.

References.—2nd Interim Report: Page 30, para. 53; page 34, paras. 60 to 65; page 89.

Appendix to 2nd Interim Report, Vol. II.: Pages 12 and 836 to 857.

Further investigations (Chart 30) subsequently carried out by Dr. Stanley Griffith demonstrated that the culture obtained from the original material contained both bovine and human tubercle bacilli, whereas cultures obtained after passage through the calf were composed of bovine tubercle bacilli only. The irregularity in the virulence of the virus was due, therefore, to the presence in the mesenteric glands of the man, as also in the culture obtained from them, of human as well as bovine tubercle bacilli; the former bacilli were eliminated from the culture by passage through the body of the calf, and a pure culture of bovine tubercle bacilli remained.

References.—Final Report: Pages 14 and 15.

Appendix to Final Report, Vol. III.: "Investigation of Certain Human Viruses of Irregular Type."

PASSAGE EXPERIMENTS IN CALVES WITH THE MIXED CULTURES DERIVED FROM VIRUS H.13."A.D." (CALF 301) AND VIRUS H.49."T.C." (MESENTERIC GLAND)

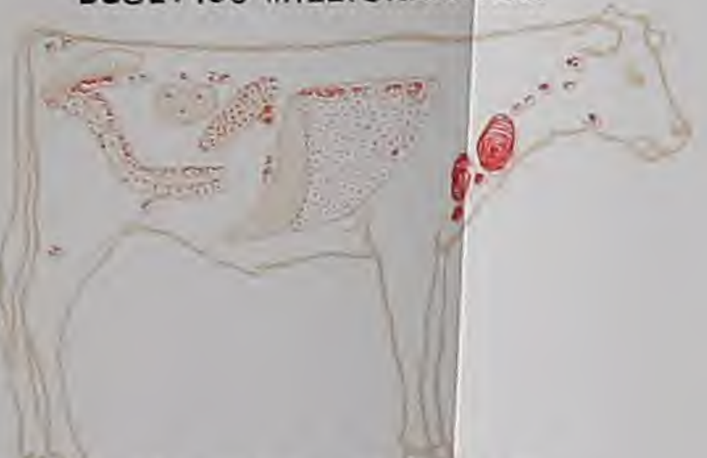
THE ORIGINAL CULTURES WERE EUGONIC; THE SECOND PASSAGE CULTURES WERE DYSGONIC.

VIRUS H.13."A.D."

The culture derived from Calf 301 was inoculated on March 23, 1907, when it had been 3⅙ years in artificial cultivation

1175. SUBCUT.

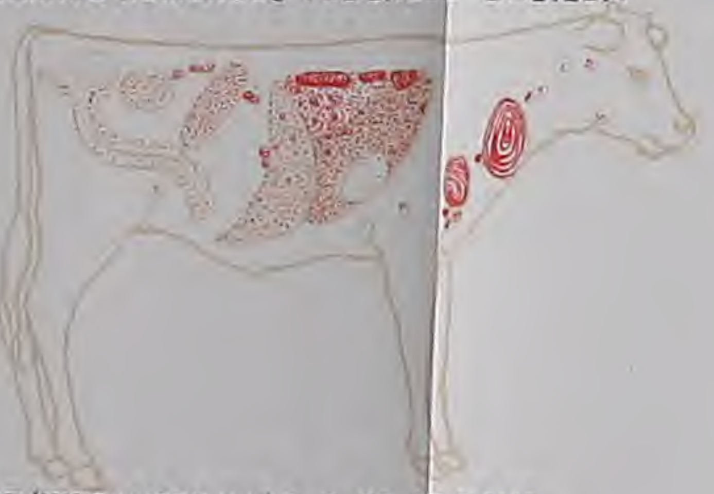
DOSE: 100 MILLIGRAMMES.



KILLED (WHEN WELL) 12.6.07. 81 DAYS

1237. SUBCUT.

DOSE: 20 C.C. OF EMULSION OF PRESCAPULAR GLAND, CONTAINING NUMEROUS TUBERCLE BACILLI.



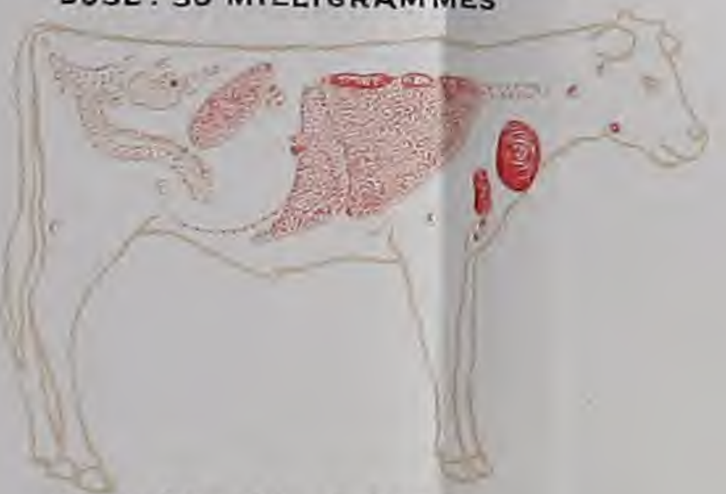
KILLED (WHEN VERY ILL) 3.8.07 52 DAYS.

CULTURE

Derived from the Bronchial gland of Calf 1237. Inoculated on Dec. 30, 1907, after 149 days artificial cultivation.

1327. SUBCUT.

DOSE: 50 MILLIGRAMMES



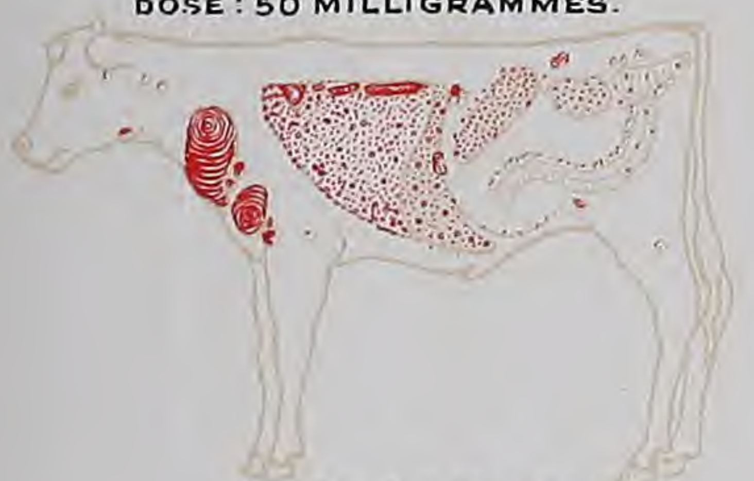
DIED: 26.1.08. 27 DAYS.

VIRUS H.49."T.C."

The culture derived from the human mesenteric gland was inoculated on April 20. 1907, when it had been 2 years in artificial cultivation.

1213. SUBCUT.

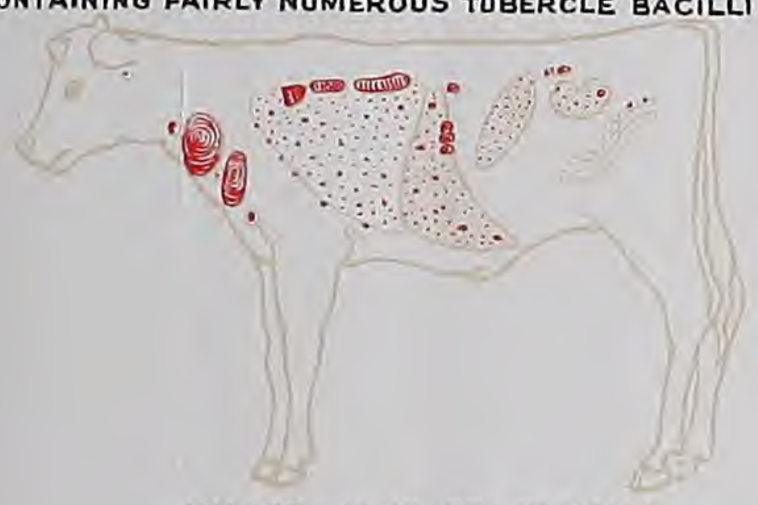
DOSE: 50 MILLIGRAMMES.



KILLED: 9.7.07. 80 DAYS
(WHEN IN MODERATE HEALTH)

1263. SUBCUT.

DOSE: 10 C.C. OF EMULSION OF PRESCAPULAR GLAND, CONTAINING FAIRLY NUMEROUS TUBERCLE BACILLI.



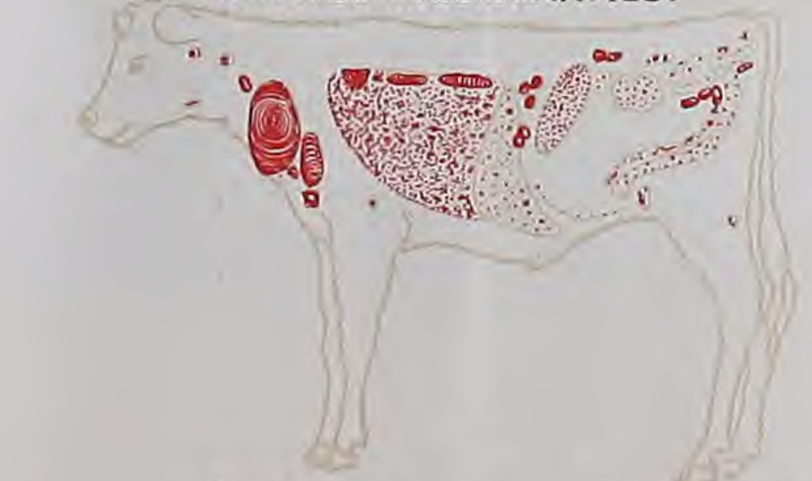
KILLED: 8.10.07. 91 DAYS.

CULTURE

Derived from Mediastinal gland of Calf 1263. Inoculated on Dec. 30. 1907, after 83 days artificial cultivation

1347. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED (WHEN ILL) 20.2.08. 52 DAYS

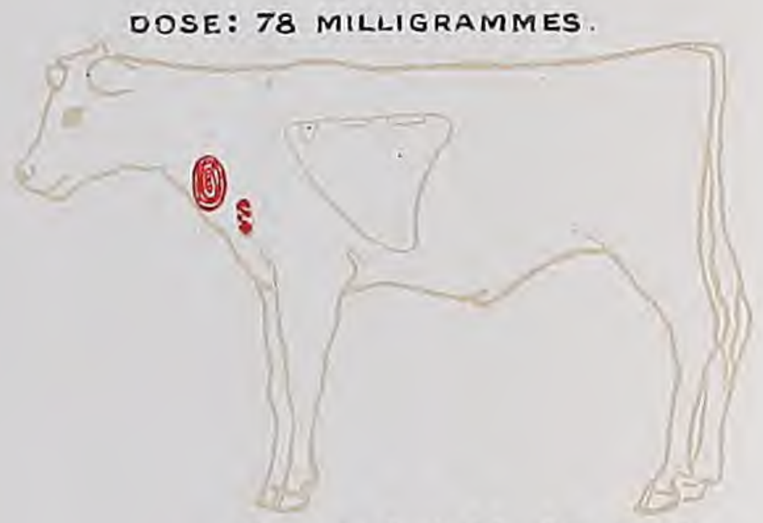
INOCULATION EXPERIMENTS ON CALVES WITH CULTURES RAISED FROM SINGLE COLONIES OBTAINED BY MEANS OF PLATE CULTURES FROM THE "CALF 301" STRAIN OF H.13.A.D. & THE "MESENTERIC GLAND" STRAIN OF H 49 "T.C."

VIRUS H.13."A.D."

Calf inoculated on Jan. 16. 1908, with the Eugonic culture from Colony Nº 6. (G.P. 2605)

1343. SUBCUT.

DOSE: 78 MILLIGRAMMES.

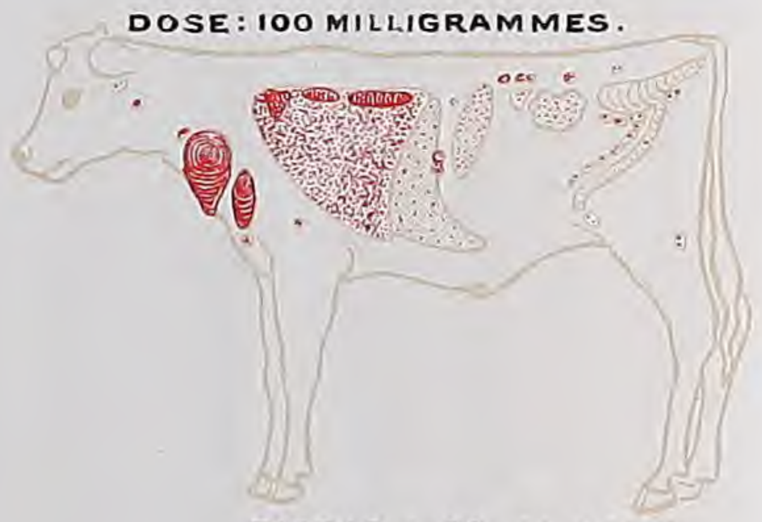


KILLED: 9.4.08. 84 DAYS.

Calf inoculated on Nov. 12. 1907, with the Eugonic culture from Colony Nº 2 (G.P. 2491)

1301. SUBCUT.

DOSE: 100 MILLIGRAMMES.

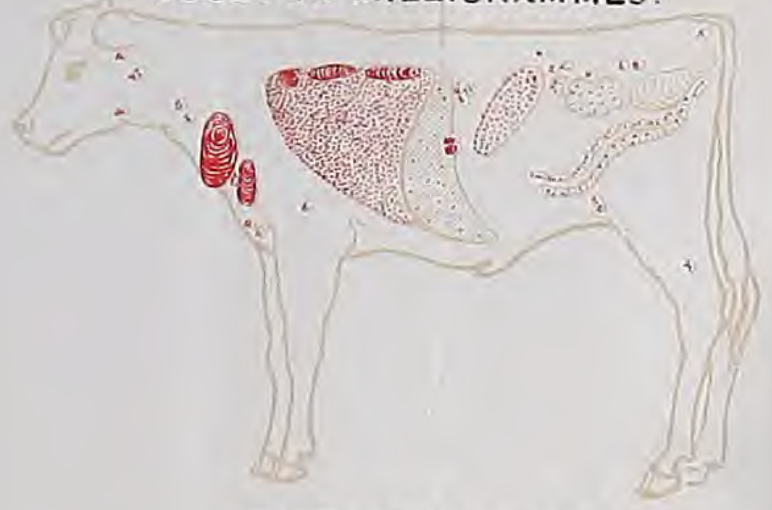


DIED: 2.1.08. 51 DAYS.

Calf inoculated on Nov. 12. 1907, with the Dysgonic culture from Colony Nº 5 (G.P. 2491.)

1309. SUBCUT.

DOSE: 44 MILLIGRAMMES.



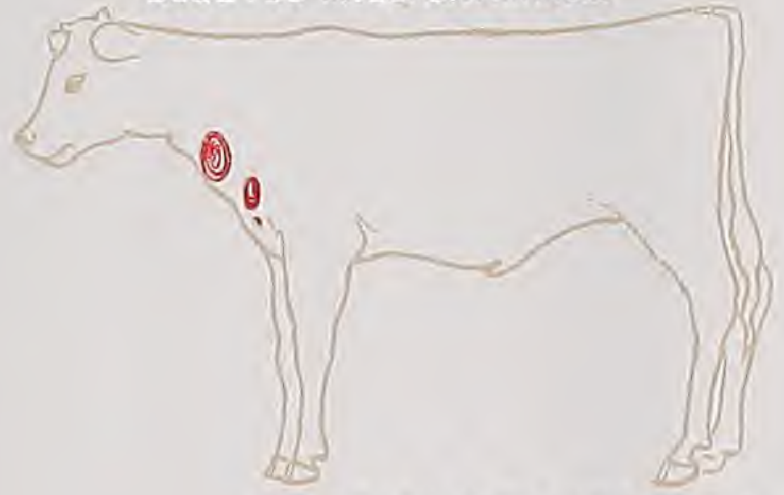
DIED: 12.12.07. 30 DAYS.

VIRUS H.49."T.C."

Calf inoculated on May 18. 1908, with the Eugonic culture from Colony Nº 7 (G.P. 2918).

1415. SUBCUT.

DOSE: 50 MILLIGRAMMES

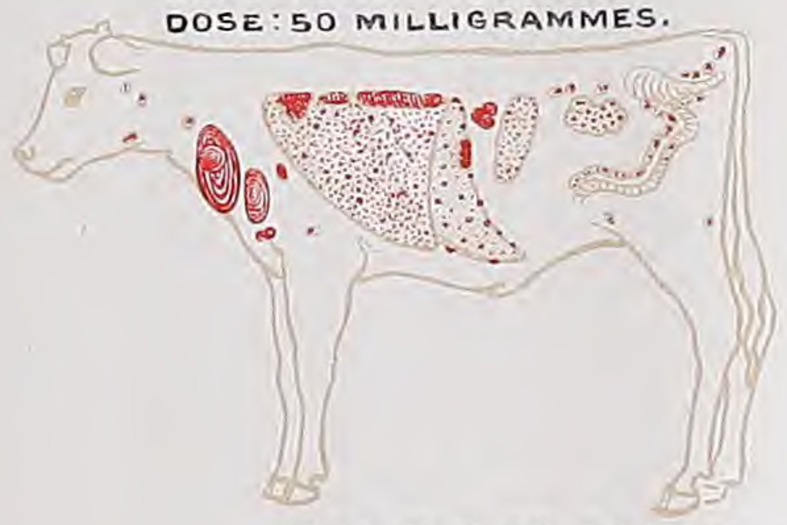


KILLED: 28.8.08. 102 DAYS.

Calf inoculated on May 13. 1908, with the Eugonic culture from Colony Nº 3 (G.P. 2918)

1387. SUBCUT.

DOSE: 50 MILLIGRAMMES.

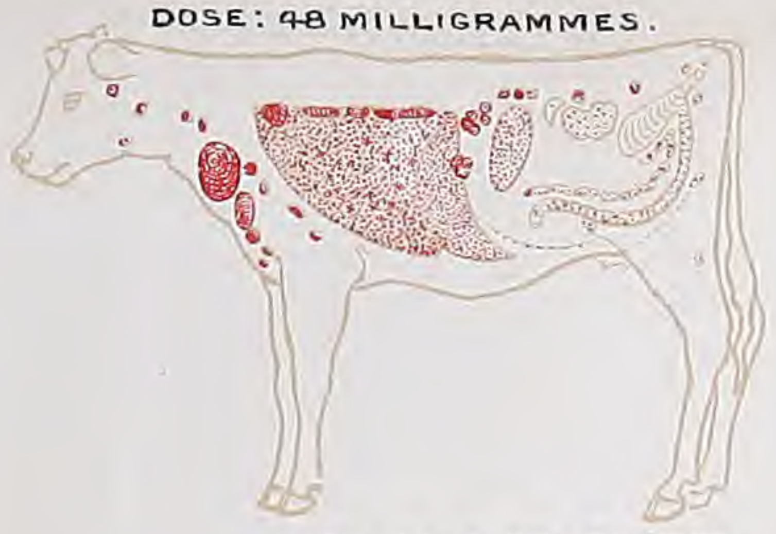


DIED: 10.7.08. 58 DAYS.

Calf inoculated on Nov. 12. 1908 with the Dysgonic culture from Colony Nº 17. (G.P. 2918)

1501. SUBCUT.

DOSE: 48 MILLIGRAMMES.



DIED: 27.12.08. 45 DAYS.

CHART 30.

Showing "Further Investigations" with the cultures derived from Virus "H.13.A.D." (Calf 301) and Virus H. 49. "T.C." (mesenteric gland).

(*See* Charts 25 and 25A and Chart 29.)

The experiments here illustrated demonstrate that these cultures, which have the cultural characters of the human tubercle bacillus and the virulence of the bovine tubercle bacillus, are composed of a mixture of bovine and human tubercle bacilli, each element of the mixture having eventually been isolated by appropriate means. (*See* Chart.)

References.—Final Report: Pages 14 and 15.

Appendix to Final Report, Vol. III.: "Investigation of Certain Human Viruses of Irregular Type."

INOCULATIONS IN CALVES WITH THE CULTURES FROM THE ORIGINAL MATERIAL: H. 60."W.B."

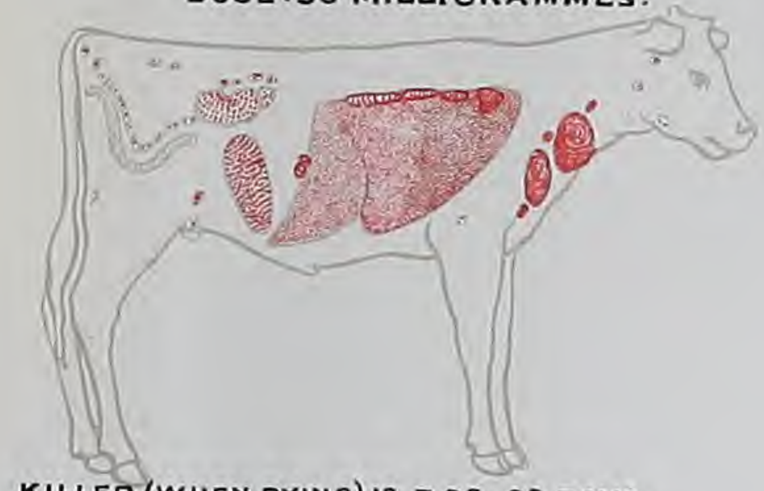
ALL THE STRAINS WERE EUGONIC.

BRONCHIAL GLANDS.

The strain was derived from the human bronchial glands through G.P. 1825 and was inoculated on June 15, 1906. when it had been 72 days in artificial cultivation.

1099. SUBCUT.

DOSE: 50 MILLIGRAMMES.



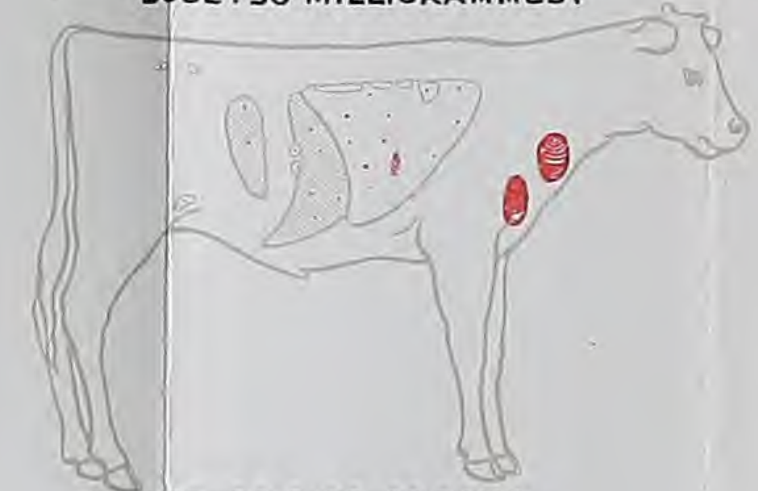
KILLED (WHEN DYING) 13.7.06. 28 DAYS.

LUNG.

The strain was derived from the human Lung through G.P. 1827 and was inoculated on May 22, 1906. when it had been 60 days in artificial cultivation.

1081. SUBCUT.

DOSE: 50 MILLIGRAMMES.



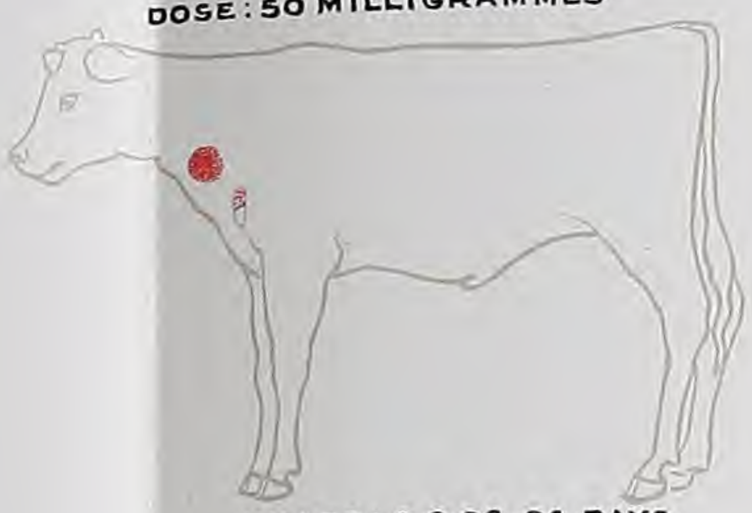
KILLED 20.8.06. 90 DAYS.

MESENTERIC GLANDS.

The strain was derived from the human mesenteric glands through G.P. 1829 and was inoculated on May 1, 1906 when it had been 41 days in artificial cultivation.

1049. SUBCUT.

DOSE: 50 MILLIGRAMMES



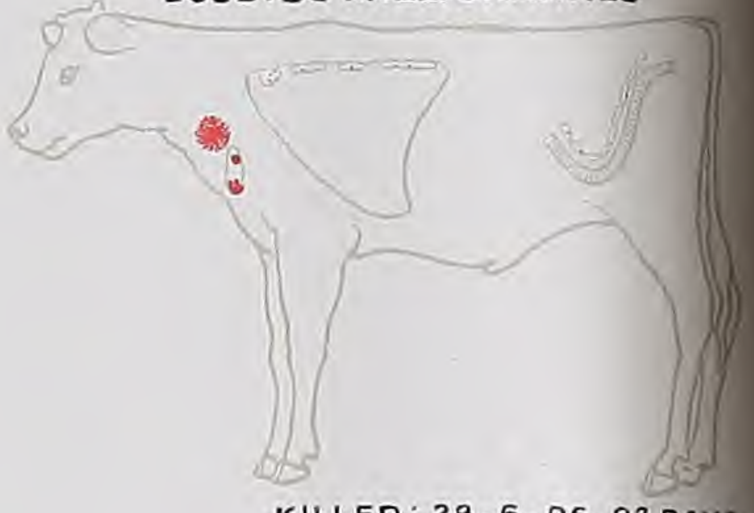
KILLED: 3.8.06. 94 DAYS.

MENINGES.

The strain was derived from the human meninges through G.P. 1832 and was inoculated on March 28, 1906. when it had been 36 days in artificial cultivation

995. SUBCUT.

DOSE: 50 MILLIGRAMMES



KILLED: 28.6.06. 92 DAYS.

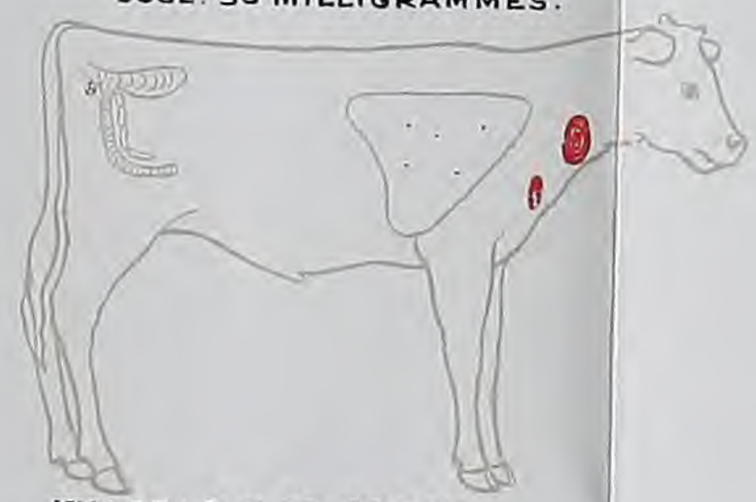
EXPERIMENTS SHOWING THE DIFFERENCE IN VIRULENCE OF TWO STRAINS ISOLATED BY PLATE CULTIVATION FROM THE BRONCHIAL GLAND STRAIN OF VIRUS H. 60."W.B."

COLONY Nº 1 STRAIN WAS EUGONIC.

Calf inoculated on July 30. 1908 with the Eugonic culture raised from Colony Nº 1 (G.P. 2489.)

1481. SUBCUT.

DOSE: 50 MILLIGRAMMES.



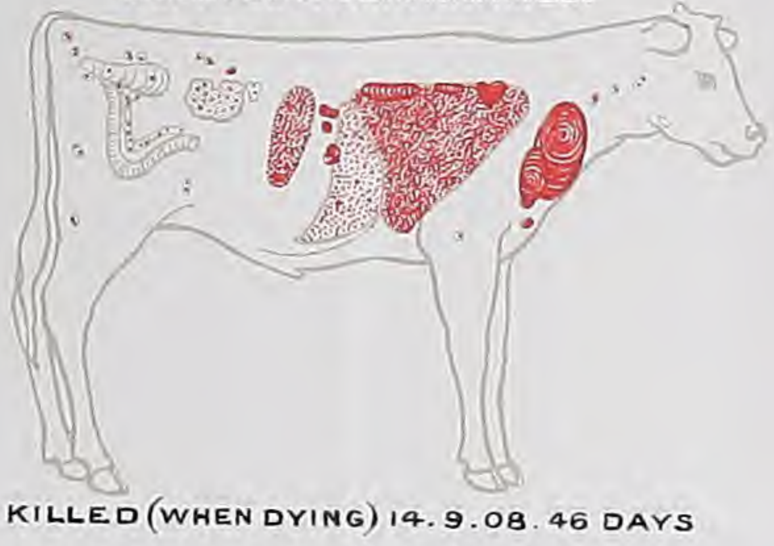
KILLED: 3.11.08. 96 DAYS.

COLONY Nº 5 STRAIN WAS DYSGONIC.

Calf inoculated on July 30. 1908 with the Dysgonic culture raised from Colony Nº 5. (G.P. 2489.)

1425. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED (WHEN DYING) 14.9.08. 46 DAYS

EXPERIMENTS ON CALVES WITH THE MIXED CULTURE DERIVED FROM VIRUS H. 90."I.P." (RETROPERITONEAL GLAND).

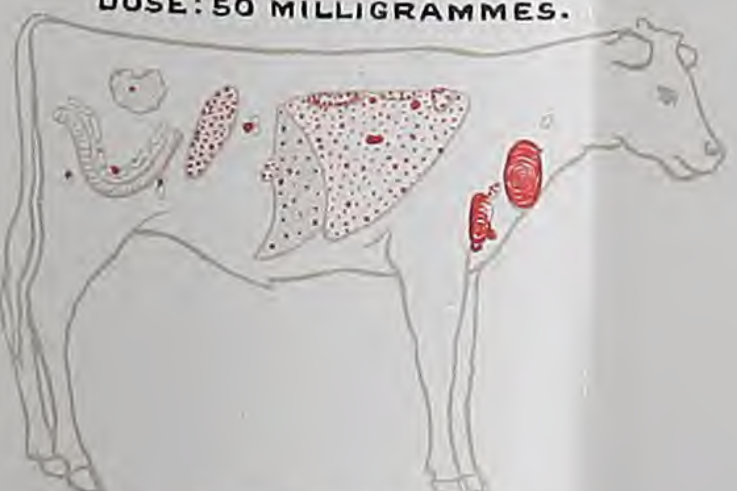
THE ORIGINAL CULTURE WAS EUGONIC.

THE FIRST PASSAGE CULTURES WERE DYSGONIC.

The strain was derived from the human retro-peritoneal gland direct; it was inoculated on July 8, 1908. when it had been 340 days in artificial cultivation.

1383. SUBCUT.

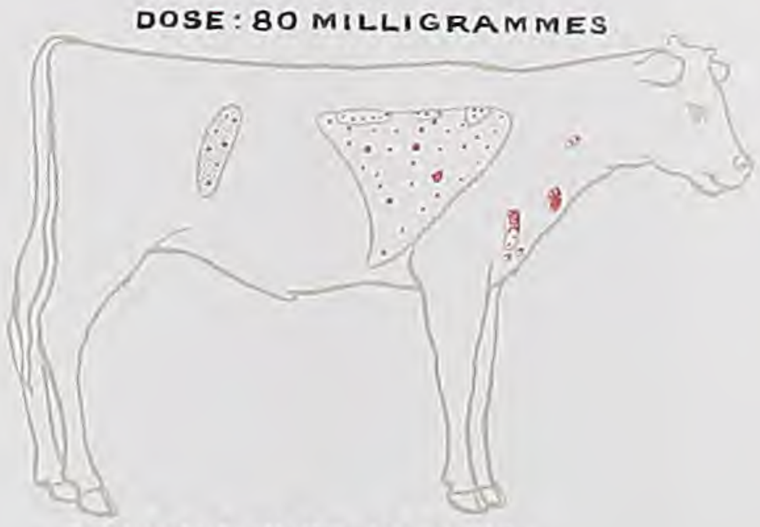
DOSE: 50 MILLIGRAMMES.



KILLED: 8.10.08. 92 DAYS

1471. SUBCUT.

DOSE: 80 MILLIGRAMMES

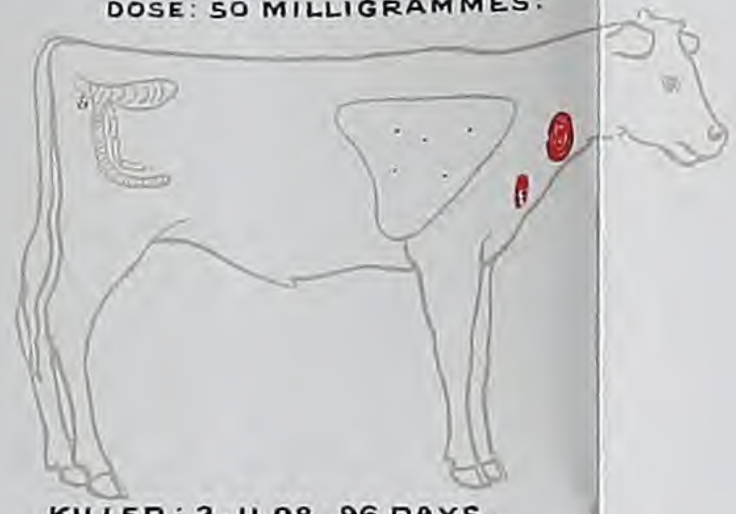


KILLED: 6.10.08. 90 DAYS.

from Colony No 1 (G.P. 2489.)

1481. SUBCUT.

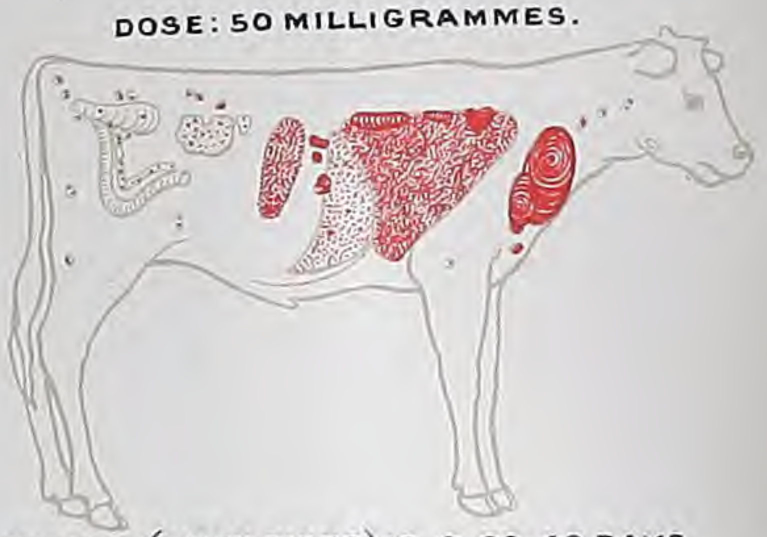
DOSE: 50 MILLIGRAMMES.



KILLED: 3.11.08. 96 DAYS.

1425. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED (WHEN DYING) 14.9.08. 46 DAYS

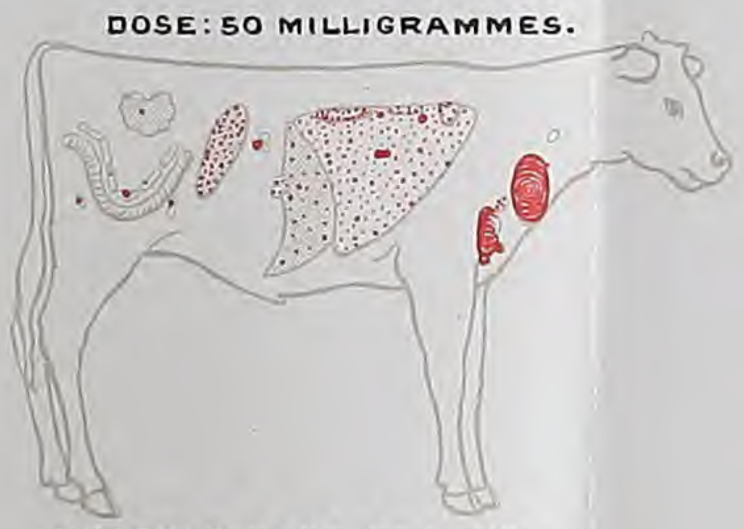
EXPERIMENTS ON CALVES WITH THE MIXED CULTURE DERIVED FROM VIRUS H.90."I.P." (RETROPERITONEAL GLAND).

THE ORIGINAL CULTURE WAS EUGONIC. THE FIRST PASSAGE CULTURES WERE DYSGONIC.

The strain was derived from the human retro-peritoneal gland direct; it was inoculated on July 8, 1908. when it had been 340 days in artificial cultivation.

1383. SUBCUT.

DOSE: 50 MILLIGRAMMES.



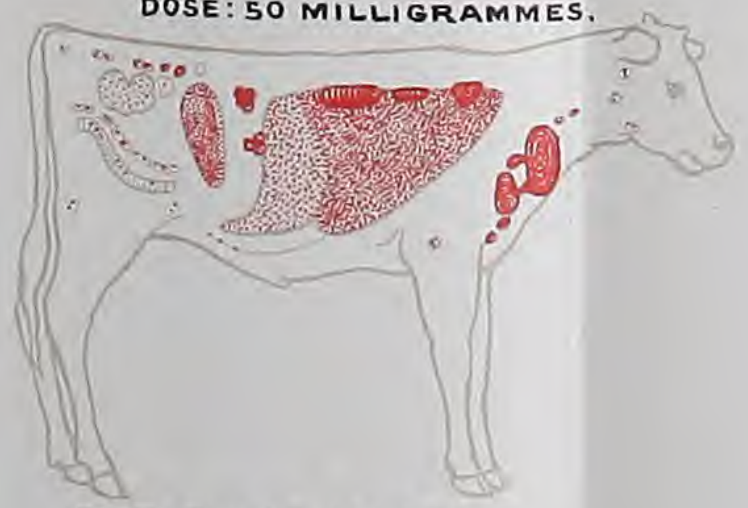
KILLED: 8.10.08. 92 DAYS

CULTURE.

Derived from the pudic gland of Calf 1383. Inoculated on November 25, 1908. Duration of artificial cultivation: 48 days.

1489. SUBCUT.

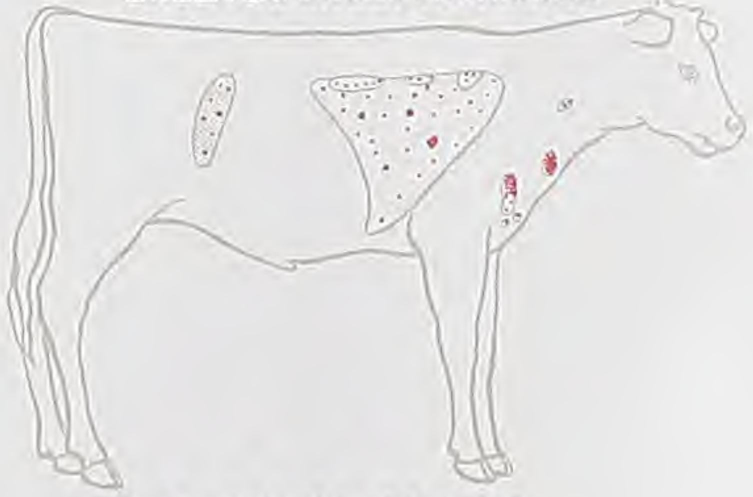
DOSE: 50 MILLIGRAMMES.



DIED, 6.1.09. 42 DAYS.

1471. SUBCUT.

DOSE: 80 MILLIGRAMMES



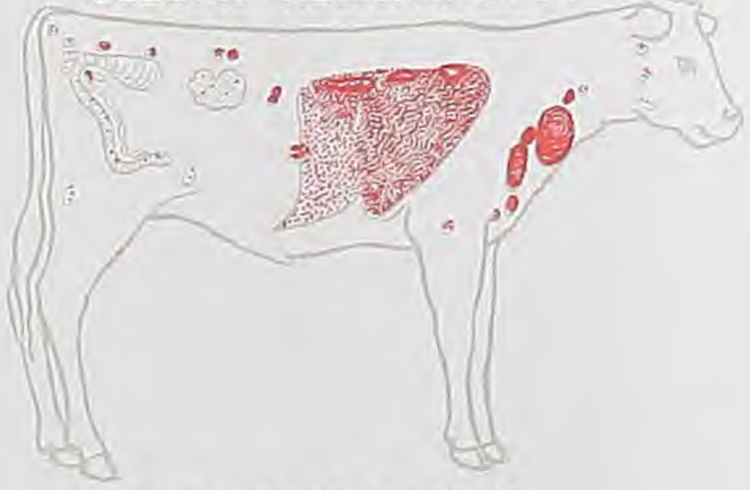
KILLED: 6.10.08. 90 DAYS.

CULTURE.

Derived from the bronchial gland of Calf 1471. Inoculated on November 23, 1908. Duration of artificial cultivation: 48 days.

1493. SUBCUT.

DOSE: 45 MILLIGRAMMES.



DIED 14.12.08. 21 DAYS.

CHART 31.

The Special Investigation of the two Cultures H. 60. "W.B." (Bronchial Gland) and H. 90. "I.P." (Retroperitoneal Gland), isolated since the issue of the 2nd Interim Report, which exhibited the features of the two cultures H. 13. "A.D." (Calf 301) and H. 49. "T.C." (Mesenteric Gland).

VIRUS H. 60. "W.B."

Nature of case : *General Miliary Tuberculosis.*

Age of patient : 4 years, 7 months.

Material used (*post-mortem*) : Bronchial Glands, Lung, Mesenteric Glands, Meninges.

Explanation of Chart.—*Cultures* were isolated from each of the above-mentioned human tissues and inoculated (Chart 31) into calves. All the strains of culture were eugonic, and three were shown to be slightly virulent for the calf ; the fourth was virulent for the calf (Calf 1099).

By a method of plate cultivation, a eugonic slightly virulent culture and a dysgonic virulent strain of culture were obtained from the bronchial gland strain (*see* Chart 31, Calves 1481 and 1485), which was thus shown to be a mixture of bovine and human tubercle bacilli.

References.—Final Report : Pages 14 and 15.

Appendix to Final Report, Vol. I. : Pages 11, 16, 109 and 275 ; Vol. III. : "The Investigation of Certain Human Viruses of Irregular Type."

VIRUS H. 90. "I.P."

Nature of case : *Tuberculosis of Mesenteric and Retroperitoneal Glands* (death from pneumonia).

Age of patient : 70 years.

Material used (*post-mortem*) : Mesenteric and Retroperitoneal Glands.

Explanation of Chart : *Cultures* were isolated from each of the above-mentioned human tissues ; the culture from the mesenteric glands was eugonic and slightly virulent for the rabbit, and was not further tested ; the culture from the retroperitoneal glands was also eugonic but proved virulent for the rabbit.

The retroperitoneal gland was therefore tested on two calves (Nos. (1383 and 1471, Chart 31) and proved to be virulent, but less virulent than a bovine tubercle bacillus. Cultures from these calves were dysgonic and equal in virulence for the calf (Calves 1489 and 1493) to cultures of the bovine tubercle bacillus.

By a special method of cultivation there were obtained a eugonic culture which exhibited the virulence of the human tubercle bacillus, and a dysgonic culture which exhibited the virulence of the bovine tubercle bacillus.

Thus the retroperitoneal gland strain of Virus H. 90. "I.P." has been shown to be a mixture of human and bovine tubercle bacilli.

References.—Final Report : Pages 14 and 15.

Appendix to Final Report, Vol. I. : Pages 7, 15, 134, and 457 ; Vol. III. : "Investigation of Certain Human Viruses of Irregular Type."

3.

(*a.*) Two charts illustrating 14 viruses, investigated after the issue of the 2nd Interim Report, showing typical features of Group I. or Group II.

Pages 44 and 45

(*b.*) Seven charts illustrating the investigation by Dr. A. S. Griffith of 19 lupus viruses, together with one lupus virus (H. 53) investigated by Dr. Cobbett and re-investigated by Dr. Griffith.

Pages 46 to 52

(*c.*) Five charts illustrating the investigation of four equine viruses.

Pages 53 to 57

INOCULATIONS IN CALVES WITH CULTURES FROM THE SIX VIRULENT HUMAN VIRUSES.

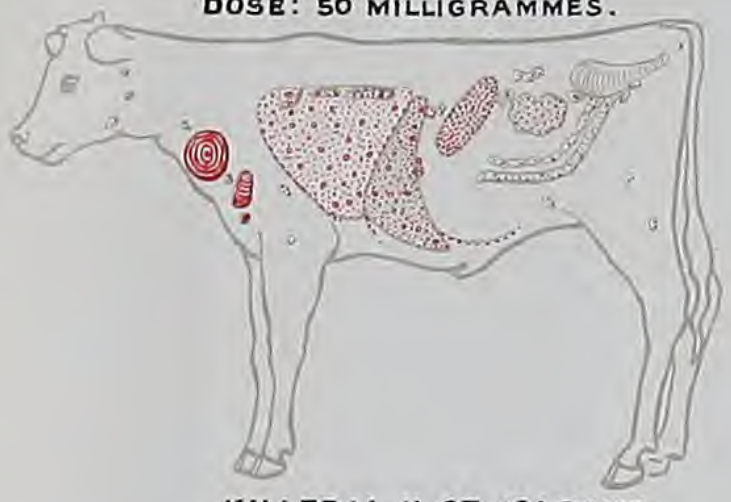
VIRUS H. 69. "F.K."

(PRIMARY ABDOMINAL TUBERCULOSIS)

The strain was derived from the human mesenteric glands through G.P. 1962, and was inoculated on July 23. 1907. when it had been 259 days in artificial cultivation.

1249. SUBCUT.

DOSE: 50 MILLIGRAMMES.

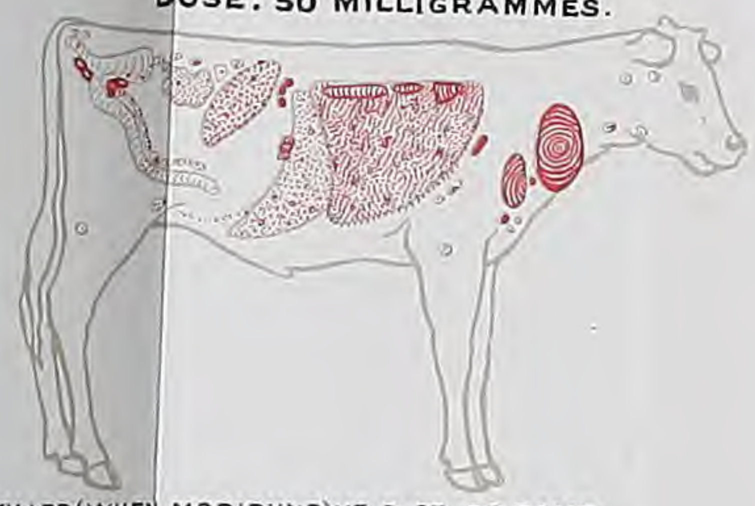


KILLED: 1. 11. 07. 101 DAYS.

The strain was derived from the meninges of the brain through G.P. 1924, and was inoculated on July 23, 1907. when it had been 389 days in artificial cultivation.

1267. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED (WHEN MORIBUND): 17. 9. 07. 56 DAYS.

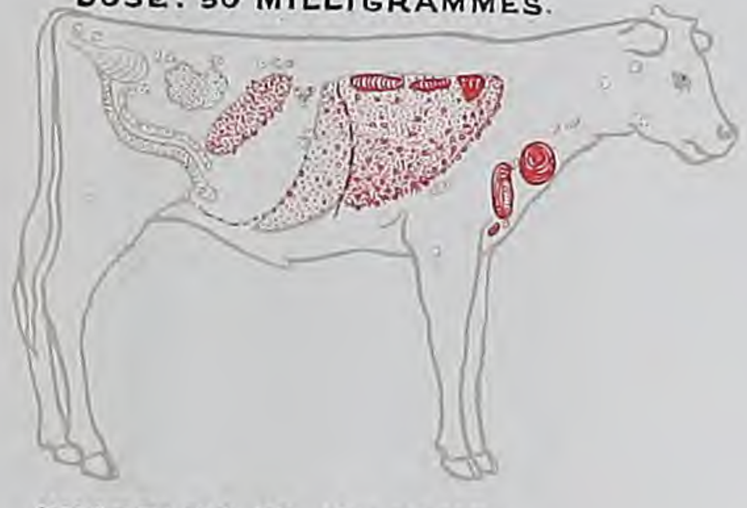
VIRUS H. 89. "W.I."

(PRIMARY ABDOMINAL TUBERCULOSIS)

The strain was derived from the human lung through G.P. 2474 and was inoculated on October 25, 1907. when it had been 123 days in artificial cultivation.

1275. SUBCUT.

DOSE: 50 MILLIGRAMMES.

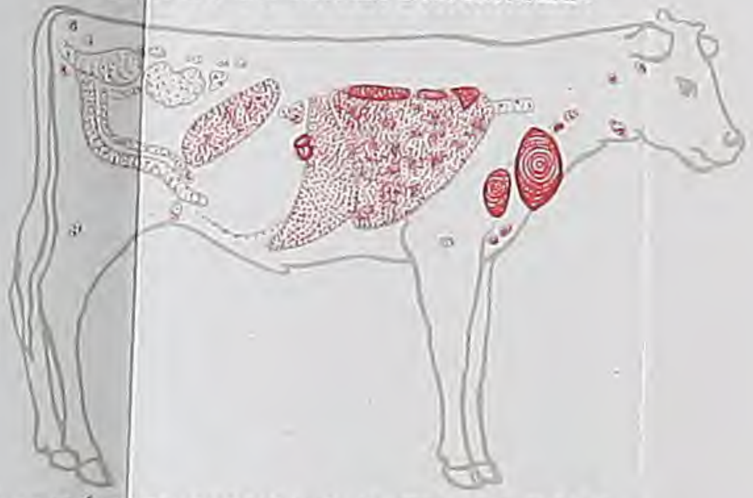


KILLED: 7.2.08. 105 DAYS.

The strain was derived from the human lung through G.P. 2474, and was inoculated on January 18. 1908. when it had been 208 days in artificial cultivation.

1351. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED (WHEN DYING): 2.3.08. 44 DAYS.

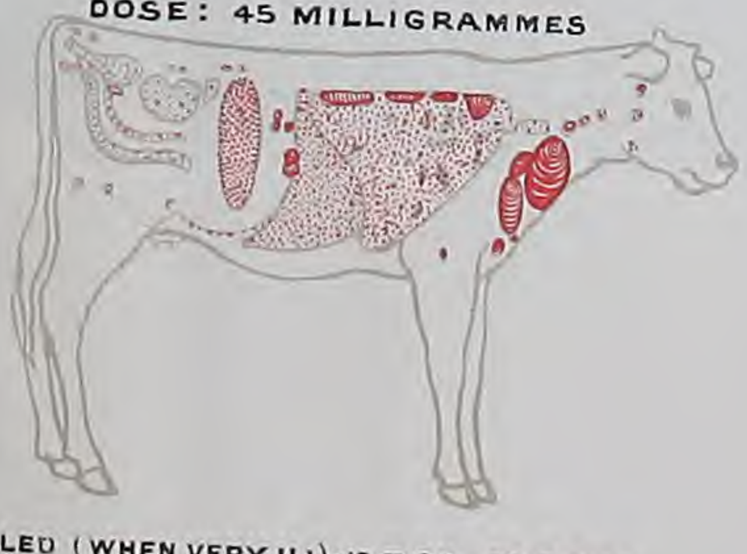
VIRUS H. 128. "D.D." (a)

(TUBERCULOSIS OF THE LUNG)

The strain was derived from human sputum, through G.P. 3584, and was inoculated on June 4, 1909 when it had been 77 days in artificial cultivation.

1567. SUBCUT.

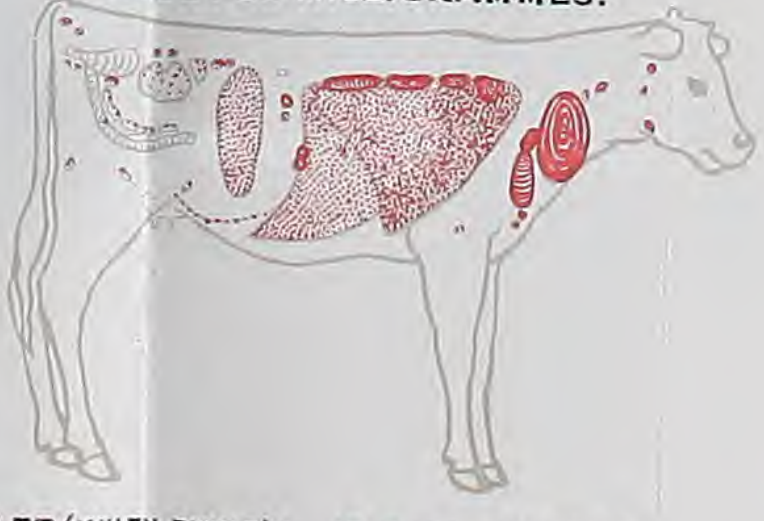
DOSE: 45 MILLIGRAMMES



KILLED (WHEN VERY ILL): 16.7.09. 42 DAYS.

1579. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED (WHEN DYING): 15.7.09. 41 DAYS.

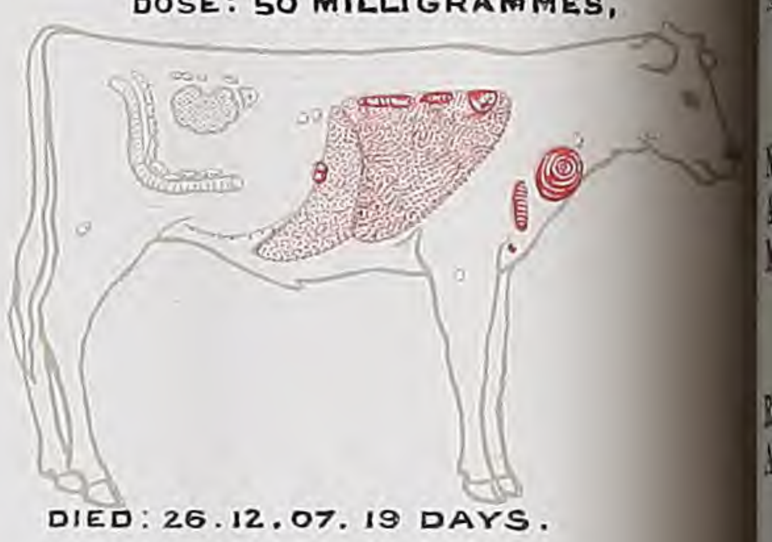
VIRUS H. 77. "R.L."

(PRIMARY ABDOMINAL TUBERCULOSIS)

The strain was derived from the human mesenteric glands direct and was inoculated on Dec. 7. 1907. when it had been 361 days in artificial cultivation.

1349. SUBCUT.

DOSE: 50 MILLIGRAMMES,



DIED: 26. 12. 07. 19 DAYS.

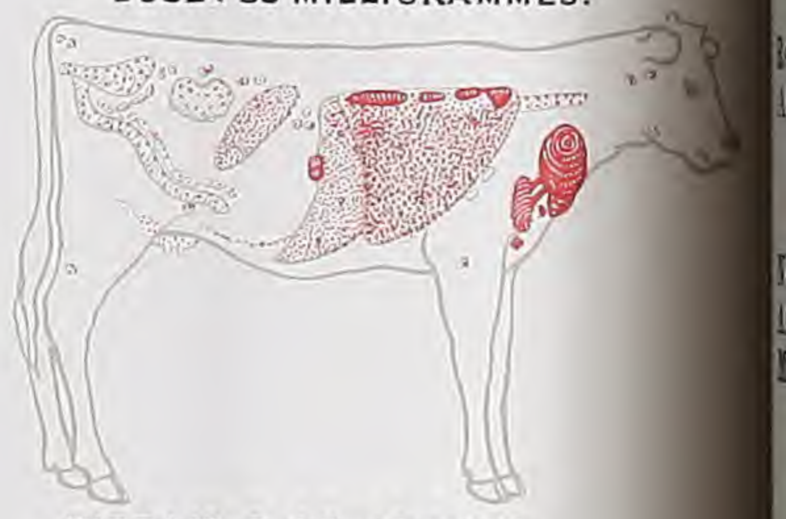
VIRUS H. 88. "E.L."

(PRIMARY ABDOMINAL TUBERCULOSIS)

The strain was derived from the human mesenteric gland and was inoculated on July 27. 1907. when it had been 85 days in artificial cultivation.

1271. SUBCUT.

DOSE: 50 MILLIGRAMMES.



DIED: 10.9.07. 45 DAYS.

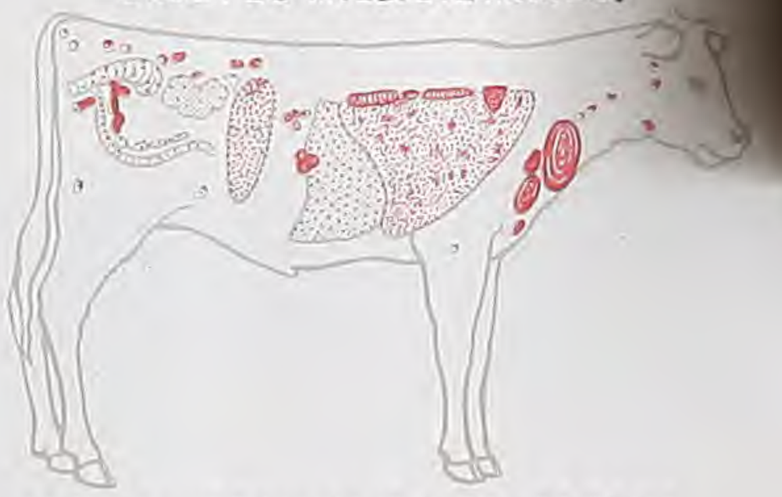
VIRUS H. 127. "R.R." (a)

(TUBERCULOSIS OF THE LUNG)

The strain was derived from human sputum, through G.P. 3474 and was inoculated on May 26, 1909. when it had been 89 days in artificial cultivation.

1585. SUBCUT.

DOSE: 50 MILLIGRAMMES,



KILLED (WHEN VERY ILL): 13.7.09. 48 DAYS.

"INOCULATIONS IN CALVES WITH CULTURES FROM THE SIX VIRULENT HUMAN VIRUSES."

All these viruses had the cultural characters of the Bovine Tubercle Bacillus.

[*See* Final Report, Appendix Vol. 1, pages 15 to 17.]

VIRUS H. 69. "F.K."

Nature of tuberculosis: *Primary Abdominal Tuberculosis.*
Age of patient: 2 years.
Material used (*post-mortem*): Mesenteric Gland and Meninges. The inoculations were carried out with *cultures* derived from these tissues.

Reference.—Final Report: Pages 11 and 13.
Appendix to Final Report, Vol. I.: Pages 112 and 313.

VIRUS H. 77. "R.L."

Nature of tuberculosis: *Primary Abdominal Tuberculosis.*
Age of patient: 13 months.
Material used (*post-mortem*): Mesenteric Glands. Calf 1349 was inoculated with a *culture* derived from this source. A *culture* obtained from the liver was also investigated. (*See* reference.)

Reference.—Final Report: Pages 11 and 13.
Appendix to Final Report, Vol. I.: Pages 120 and 361.

VIRUS H. 88. "E.L."

Nature of tuberculosis: *Primary Abdominal Tuberculosis.*
Age of patient: 8½ years.
Material used (*post-mortem*): Mesenteric Glands. The inoculations were carried out with a *culture* derived directly from the original material.

Reference.—Final Report: Pages 11 and 13.
Appendix to Final Report, Vol. I.: Pages 132 and 447.

VIRUS H. 89. "W.I."

Nature of tuberculosis: *Primary Abdominal Tuberculosis.*
Age of patient: 4 years.
Material used (*post-mortem*): Lung. Calves 1275 and 1351 were inoculated with *culture* derived from this source. A culture obtained from a mesenteric gland was also investigated. (*See* reference.)

Reference.—Final Report: Pages 11 and 13.
Appendix to Final Report, Vol. I.: Pages 133 and 451.

VIRUS H. 127. "R.R."

Nature of tuberculosis: *Tuberculosis of the Lung.*
Age of patient: 21 years.
Material used (*clinical*): Sputum: obtained on four occasions, 76, 117, and 118 days respectively, after the first. The strains of this virus are entitled H. 127. "R.R." (*a*), (*b*), (*c*), and (*d*), respectively. The calf (1585) depicted was injected with a culture of the (*a*) strain.

Reference.—Final Report: Pages 10 and 12.
Appendix to Final Report, Vol. I.: Pages 149 to 151 and 488 to 496.

VIRUS H. 128. "D.D."

Nature of tuberculosis: *Tuberculosis of the Lung.*
Age of patient: 31 years.
Material used (*clinical*): Sputum: obtained on two occasions at an interval of 118 days. The two strains of the virus thus obtained are entitled H. 28. "D.D." (*a*), and H. 128. "D.D." (*b*). The calves depicted (1567 and 1579) were injected with a culture of the (*a*) strain.

References.—Final Report: Pages 10 and 12.
Appendix to Final Report, Vol. I.: Pages 151, 152, 496, and 500.

INOCULATIONS IN CALVES WITH TISSUE EMULSIONS AND CULTURES FROM SLIGHTLY VIRULENT HUMAN VIRUS

I. TISSUE EMULSIONS.

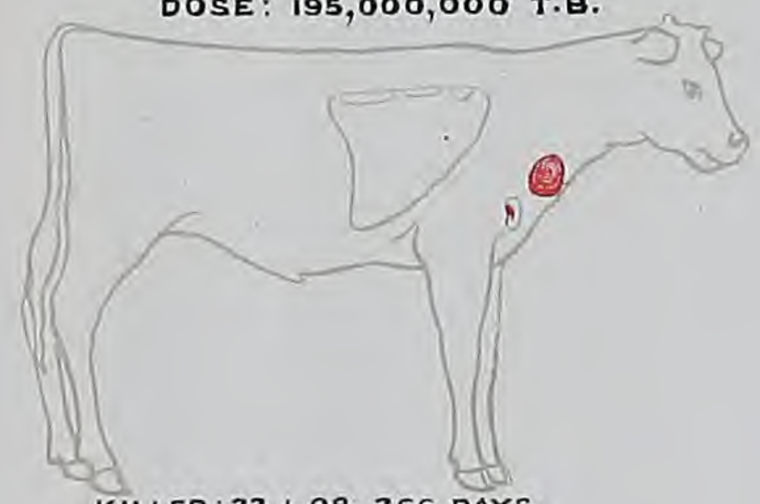
VIRUS H. 80. "D.M."

(TUBERCULOSIS OF THE LUNG)

10 cubic centimetres of an emulsion made from the Lung, estimated to contain 19.500.000 tubercle bacilli per C.C. were inoculated into each Calf on January 22. 1907.

1131. SUBCUT.

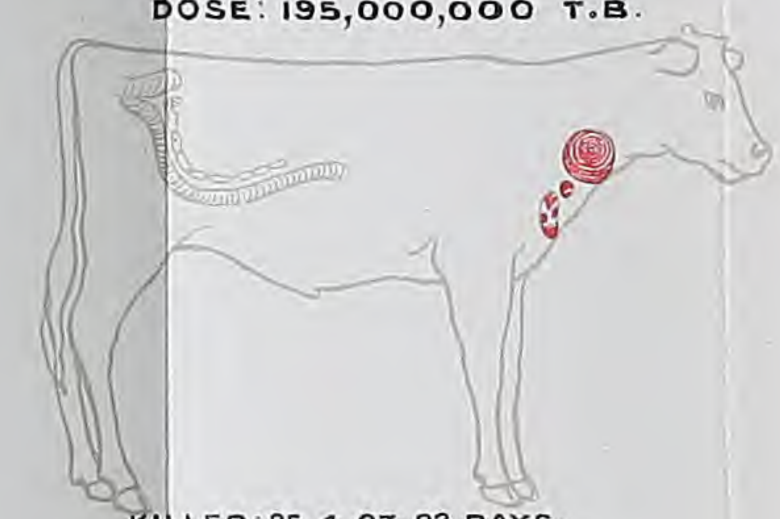
DOSE: 195,000,000 T.B.



KILLED: 23.1.08. 366 DAYS.

1141. SUBCUT.

DOSE: 195,000,000 T.B.



KILLED: 25.4.07. 93 DAYS.

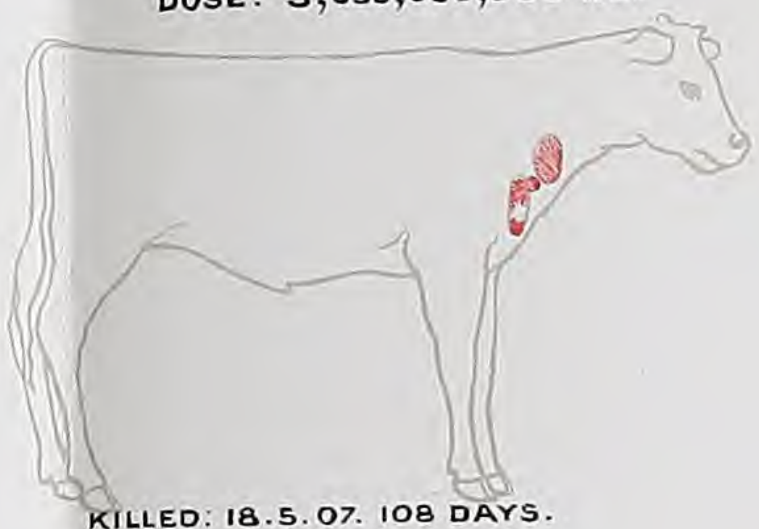
VIRUS H. 81. "P.W."

(TUBERCULOSIS OF THE LUNG.)

10 cubic centimetres of an emulsion made from the Lung, estimated to contain 303.500.000 tubercle bacilli per C.C. were inoculated into each Calf on January 30. 1907.

1129. SUBCUT.

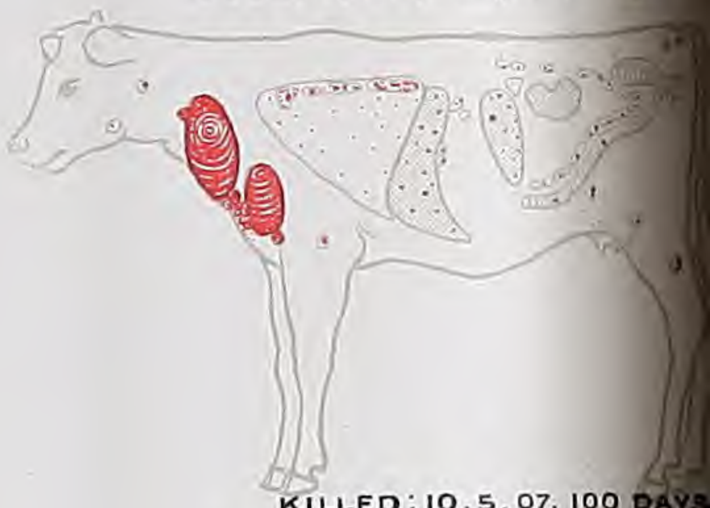
DOSE: 3,035,000,000 T.B.



KILLED: 18.5.07. 108 DAYS.

1145. SUBCUT.

DOSE: 3,035,000,000 T.B.



KILLED: 10.5.07. 100 DAYS

II. CULTURES.

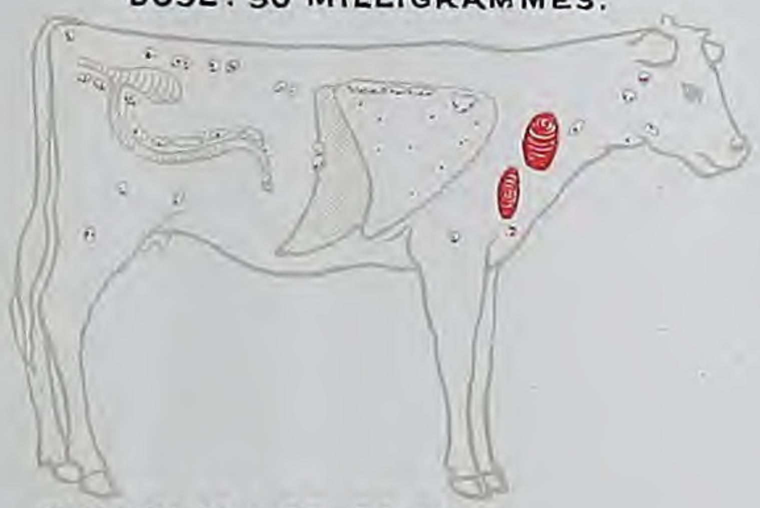
VIRUS H. 67. "E.P."

(MULTIPLE TUBERCULOSIS OF BONES.)

The strain was derived from the original material (pus from a scapular abscess) through G.P. 1914, and was inoculated on Oct. 26. 1906. when it had been 108 days in artificial cultivation.

1169. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 24.1.07. 90 DAYS.

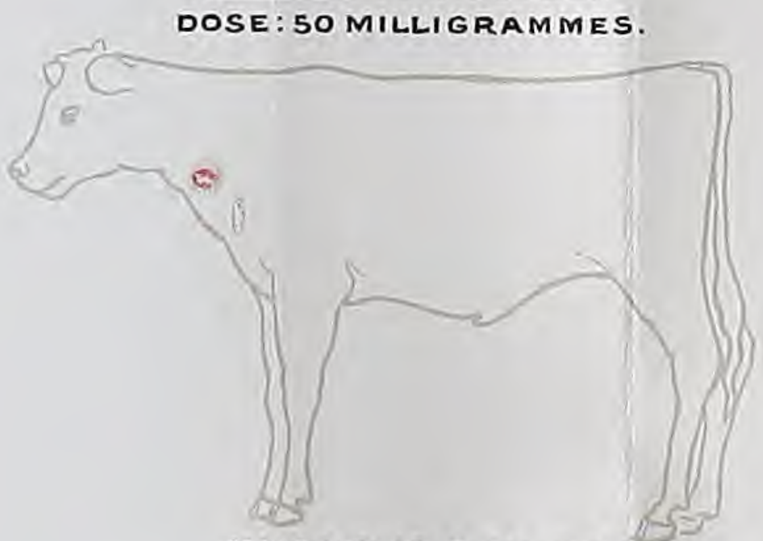
VIRUS H. 74 "D.S."

(PRIMARY ABDOMINAL TUBERCULOSIS)

The strain was derived from the human mesenteric glands direct and was inoculated on March 20, 1907. when it had been 120 days in artificial cultivation.

1181. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 16.7.07. 118 DAYS.

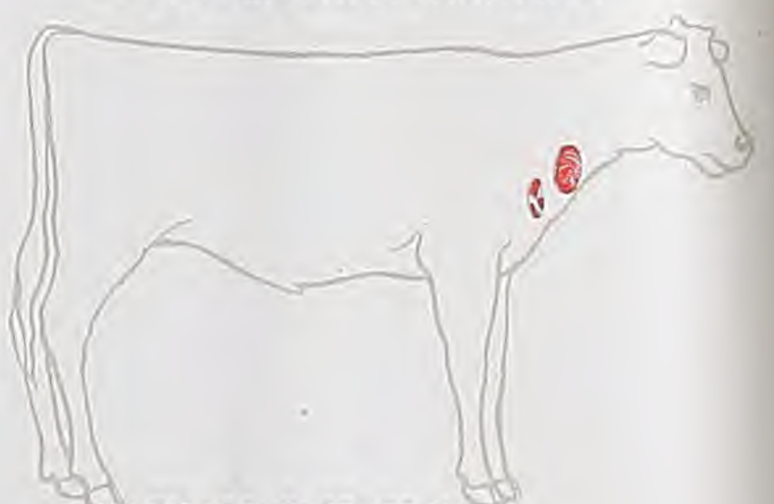
VIRUS H. 75. "A.H."

(PRIMARY ABDOMINAL TUBERCULOSIS)

The strain was derived from the human peritoneal fluid through G.P. 2018. and was inoculated on March 19, 1907, when it had been 99 days in artificial cultivation.

1195. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 24.6.07. 97 DAYS.

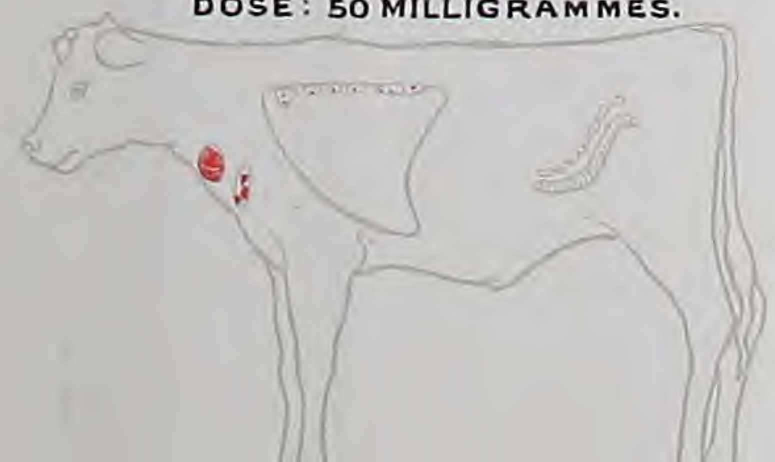
VIRUS H. 78. "O.D."

(GENERAL TUBERCULOSIS)

The strain was derived from the meninges of the brain through G.P. 2122. and was inoculated on March 21, 1907, when it had been 77 days in artificial cultivation.

1179. SUBCUT.

DOSE: 50 MILLIGRAMMES.



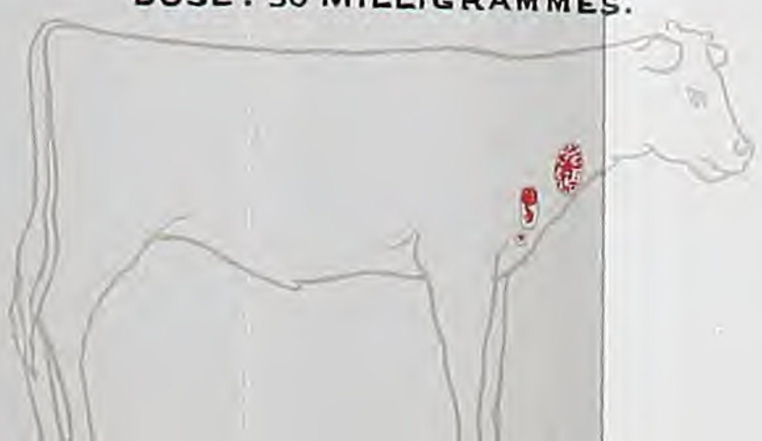
VIRUS H. 80. "D.M."

(TUBERCULOSIS OF THE LUNG)

The strain was derived from the human Lung direct, and was inoculated on April 12, 1907. when it had been 81 days in artificial cultivation.

1209. SUBCUT.

DOSE: 50 MILLIGRAMMES.



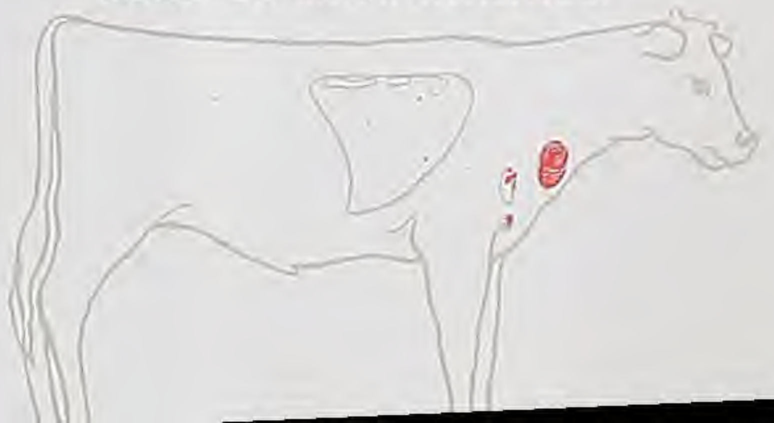
VIRUS H. 81. "P.W."

(TUBERCULOSIS OF THE LUNG)

The strain was derived from the human lung direct and was inoculated on April 12, 1907, when it had been 72 days in artificial cultivation.

1211. SUBCUT.

DOSE: 50 MILLIGRAMMES.



II. CULTURES.

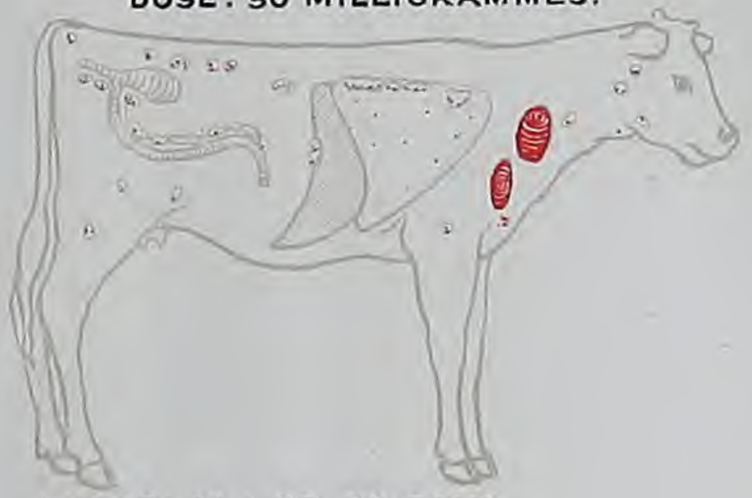
VIRUS H. 67. "E.P."

(MULTIPLE TUBERCULOSIS OF BONES.)

The strain was derived from the original material (pus from a scapular abscess) through G.P. 1914, and was inoculated on Oct. 26. 1906. when it had been 108 days in artificial cultivation.

1169. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 24. 1. 07. 90 DAYS.

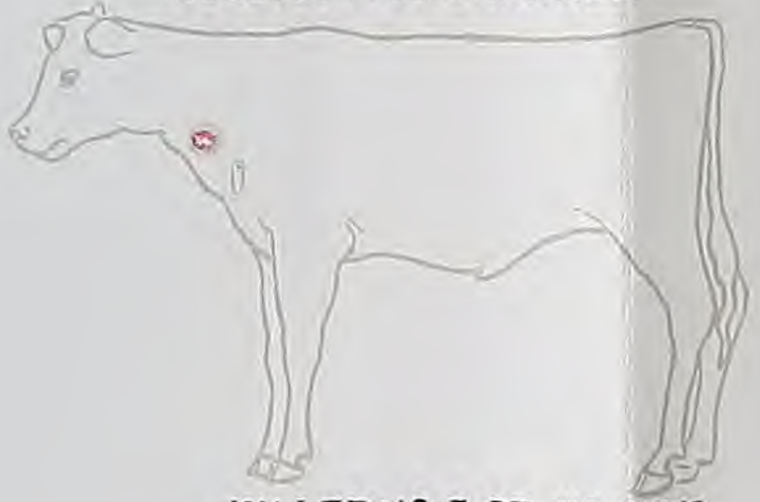
VIRUS H. 74 "D.S."

(PRIMARY ABDOMINAL TUBERCULOSIS)

The strain was derived from the human mesenteric glands direct and was inoculated on March 20, 1907. when it had been 120 days in artificial cultivation.

1181. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 16. 7. 07. 118 DAYS.

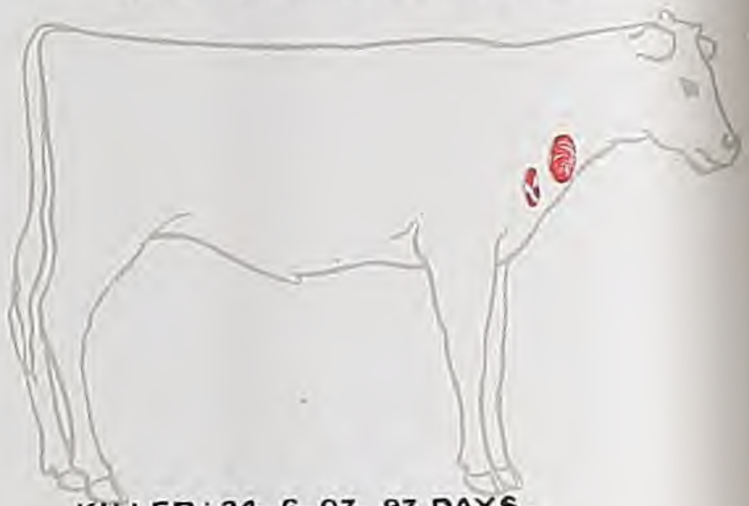
VIRUS H. 75. "A.H."

(PRIMARY ABDOMINAL TUBERCULOSIS)

The strain was derived from the human peritoneal fluid through G.P. 2018. and was inoculated on March 19, 1907, when it had been 99 days in artificial cultivation.

1195. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 24. 6. 07. 97 DAYS.

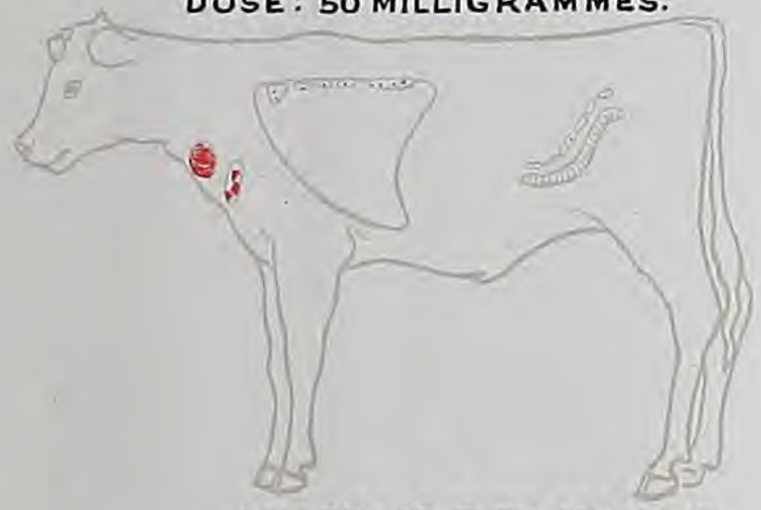
VIRUS H. 78. "O.D."

(GENERAL TUBERCULOSIS)

The strain was derived from the meninges of the brain through G.P. 2122. and was inoculated on March 21, 1907, when it had been 77 days in artificial cultivation.

1179. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 26. 7. 07. 127 DAYS.

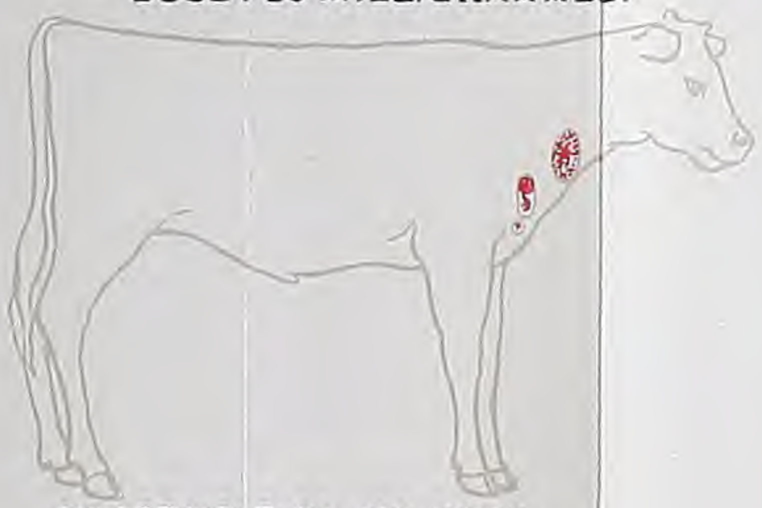
VIRUS H. 80. "D.M."

(TUBERCULOSIS OF THE LUNG)

The strain was derived from the human lung direct, and was inoculated on April 12, 1907, when it had been 81 days in artificial cultivation.

1209. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 16. 8. 07. 126 DAYS.

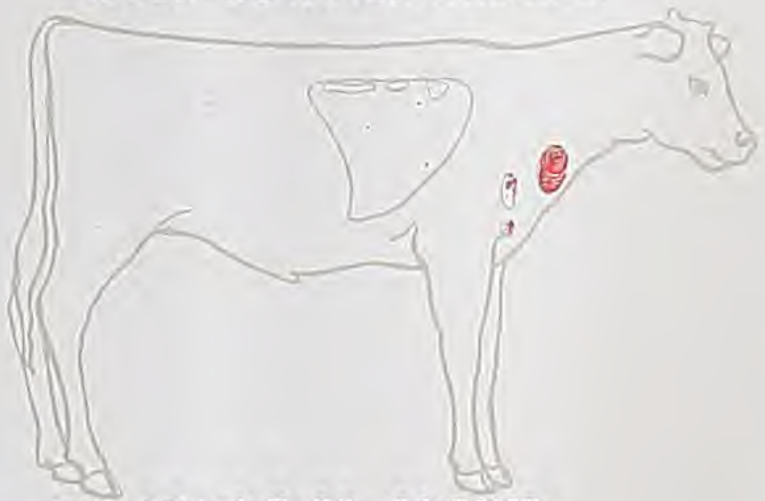
VIRUS H. 81. "P.W."

(TUBERCULOSIS OF THE LUNG)

The strain was derived from the human lung direct and was inoculated on April 12, 1907, when it had been 72 days in artificia cultivation.

1211. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 14. 8. 07. 124 DAYS.

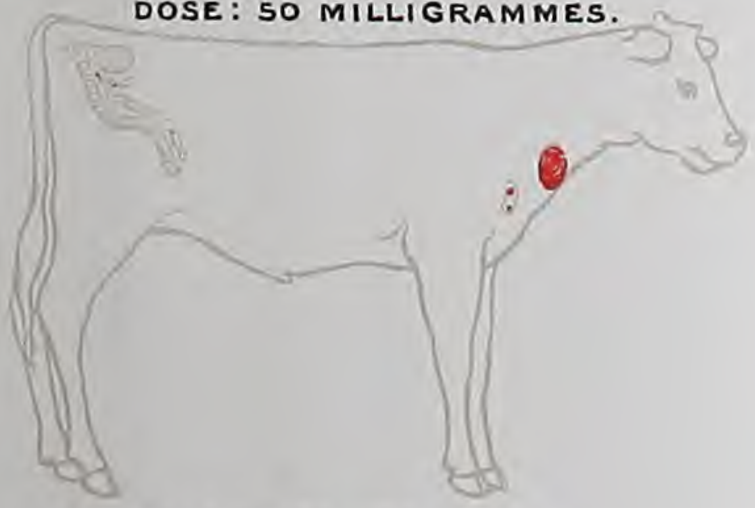
VIRUS H. 87. "W.D."

(TUBERCULOSIS OF THE SUPRARENAL BODIES.)

The strain was derived from the suprarenal body through a Guinea pig and was inoculated on June 21, 1907. when it had been 56 days in artificial cultivation.

1253. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 1. 10. 07. 102 DAYS.

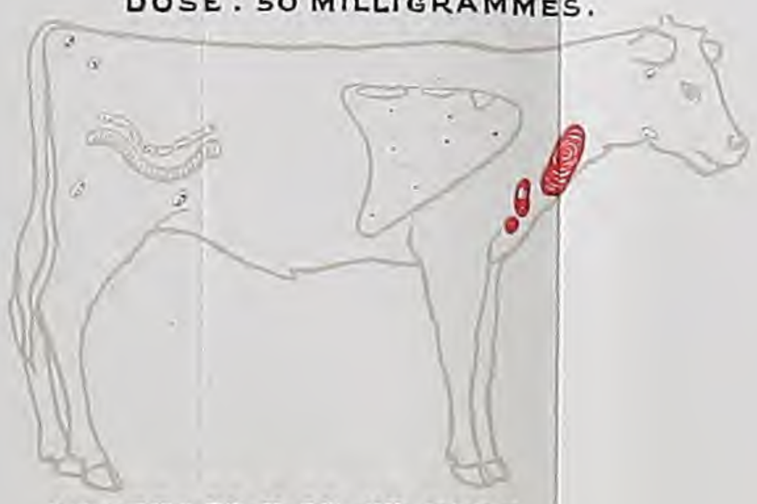
VIRUS H. 104. "E.R."

(TUBERCULOSIS OF THE LUNG WITH CALCAREOUS MESENTERIC GLANDS.)

The strain was derived from the human lung direct, and was inoculated on April 2. 1908, when it had been 51 days in artificial cultivation.

1393. SUBCUT.

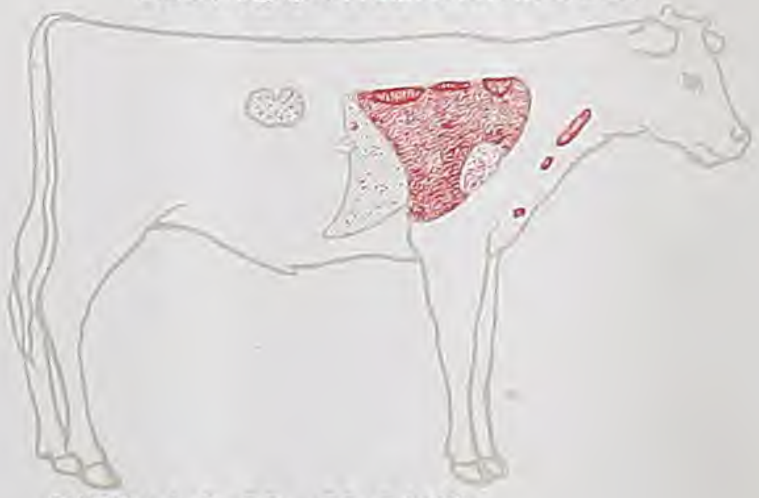
DOSE: 50 MILLIGRAMMES.



KILLED: 28. 7. 08. 117 DAYS.

1385. INTRAVENOUS.

DOSE: 5·0 MILLIGRAMMES.



DIED: 1. 5. 08. 29 DAYS.

"INOCULATIONS IN CALVES WITH TISSUE EMULSIONS AND CULTURES FROM SLIGHTLY VIRULENT HUMAN VIRUSES."

These Viruses all have the cultural characters of the Human Tubercle Bacillus.

[*See* Final Report, Appendix Vol. I., pages 15 to 17.]

VIRUS H. 67. "E.P."

Nature of tuberculosis: *Multiple Tuberculosis of Bones.*

Age of patient: 14 years.

Material used (*operation*): Pus from a scapular abscess. Calf 1169 was inoculated with a *culture* derived from the original material.

References. — Final Report: Pages 11 and 13. Appendix to Final Report, Vol. I.: Pages 111 and 289.

VIRUS H. 75. "A.H."

Nature of tuberculosis: *Primary Abdominal Tuberculosis.*

Age of patient: 7 years.

Material used (*clinical*): Peritoneal fluid. Calf 1195 was inoculated with a *culture* derived from the fluid. A *culture* was obtained *post-mortem* from the Lung and was also investigated (*see* references).

References. — Final Report: Pages 11 and 13. Appendix to Final Report, Vol. I.: Pages 118 and 353.

VIRUS H. 74. "D.S."

Nature of tuberculosis: *Primary Abdominal Tuberculosis.*

Age of patient: 7 months.

Material used (*post-mortem*): Mesenteric Gland. Calf 1181 was inoculated with a *culture* derived from the gland. *Cultures* obtained from the human Lung, Spleen, and Bronchial Glands were also investigated (*see* references).

References. — Final Report: Pages 11 and 13. Appendix to Final Report, Vol. I.: Pages 116 and 341.

VIRUS H. 78. "O.D."

Nature of tuberculosis: *General Tuberculosis probably primary in Cervical Glands.*

Age of patient: 9 months.

Material used (*post-mortem*): Meninges. Calf 1179 was inoculated with a *culture* derived from this source. *Cultures* obtained from the Lung and Bronchial Gland were also investigatated (*see* references).

References. — Final Report: Pages 10 and 12. Appendix to Final Report, Vol. I.: Pages 121 and 365.

VIRUS H. 80. "D.M."

Nature of tuberculosis: *Tuberculosis of the Lung.*

Age of patient: 33 years.

Material used (*post-mortem*). For Calves 1131 and 1141, Emulsion of the Lung. For Calf 1209, Culture obtained direct from the Lung.

References. — Final Report: Pages 10 and 12. Appendix to Final Report, Vol. I.: Pages 124 and 391.

VIRUS H. 87. "W.D."

Nature of tuberculosis: *Tuberculosis of the Suprarenal Bodies.*

Age of patient: 21 years.

Material used (*post-mortem*): Suprarenal Body. Calf 1253 was inoculated with *culture* derived from the Suprarenal.

References. — Final Report: Pages 11 and 13. Appendix to Final Report, Vol. I.: Pages 131 and 443.

VIRUS H. 81. "P.W."

Nature of tuberculosis: *Tuberculosis of the Lung.*

Age of patient: 33 years.

Material used (*post-mortem*). For Calves 1129 and 1145, Emulsion of the Lung. For Calf 1211, Culture obtained direct from the Lung.

References. — Final Report: Pages 10 and 12. Appendix to Final Report, Vol. I.: Pages 125 and 409.

VIRUS H. 104. "E.R."

Nature of tuberculosis: *Tuberculosis of the Lung with Calcareous Mesenteric Glands.*

Age of patient: 31 years.

Material used (*post-mortem*): Lung. Calves 1393 and 1385 were inoculated with a *culture* obtained direct from the Lung. A *culture* obtained from the Mesenteric Glands was also investigated (*see* references).

References. — Final Report: Pages 10 and 12. Appendix to Final Report, Vol. I.: Pages 141 and 469.

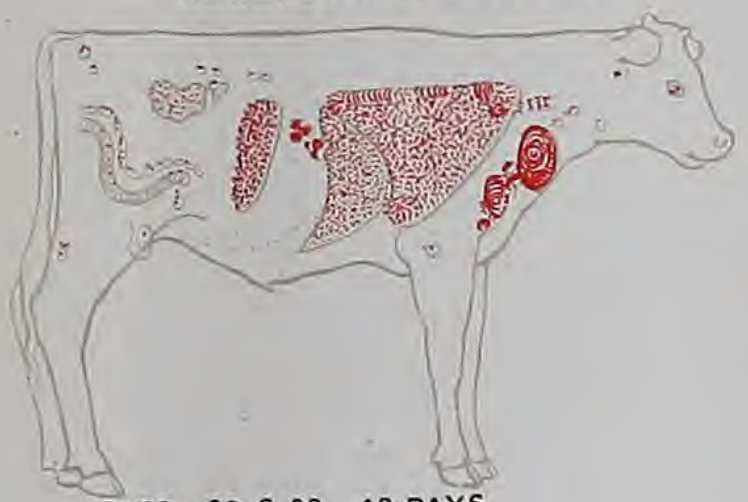
INOCULATIONS IN CALVES WITH THE LUPUS CULTURES WHICH GROW LIKE BOVINE TUBERCLE BAC

VIRUS H. 110. "J. B." *(a)*

The strain was derived from the original material through G.P. 3006. and was inoculated on July 21, 1908. when it had been 90 days in artificial cultivation.

1483. SUBCUT.

DOSE, 50 MILLIGRAMMES.

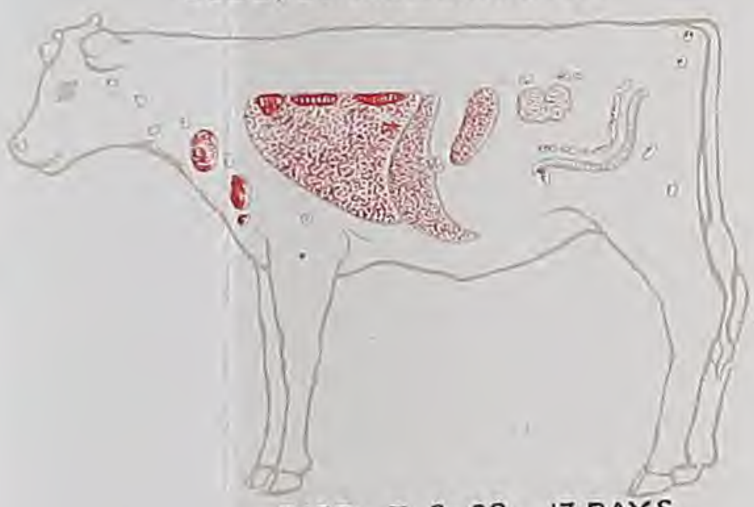


DIED. 30.8.08. 40 DAYS.

The strain was derived from the original material through G.P. 3007 and was inoculated on July, 21, 1908. when it had been 98 days in artificial cultivation.

1491. SUBCUT.

DOSE, 50 MILLIGRAMMES.



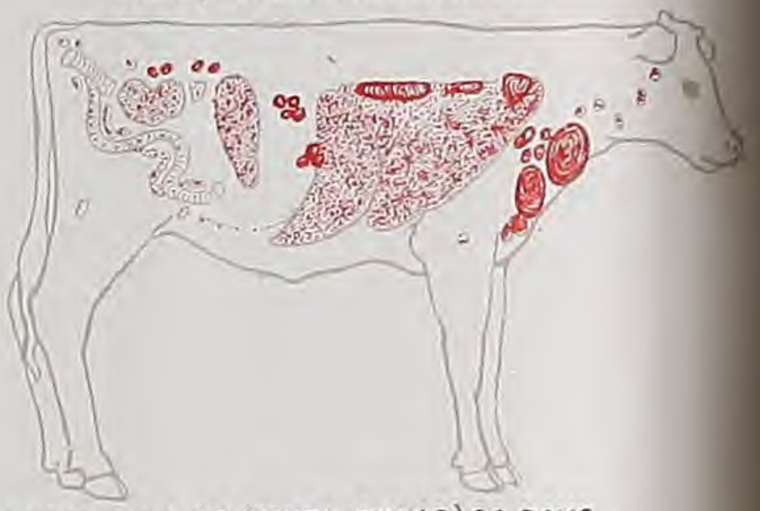
DIED. 7.8.08. 17 DAYS.

VIRUS H. 110. "J. B." *(b)*

The strain was derived from the original material through G.P. 3290. and was inoculated on December 17, 1908. when it had been 59 days in artificial cultivation.

1515. SUBCUT.

DOSE, 50 MILLIGRAMMES.



KILLED, 20.1.09. (WHEN DYING) 34 DAYS.

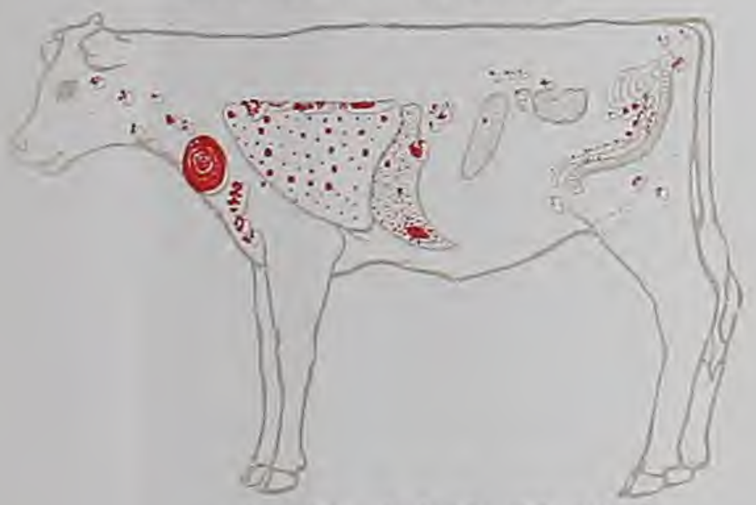
VIRUS H. 100. "R.S."

I.

The strain was derived from the original material through G.P. 2929. and was inoculated on May 19, 1908. when it had been 69 days in artificial cultivation.

1419. SUBCUT.

DOSE, 50 MILLIGRAMMES.



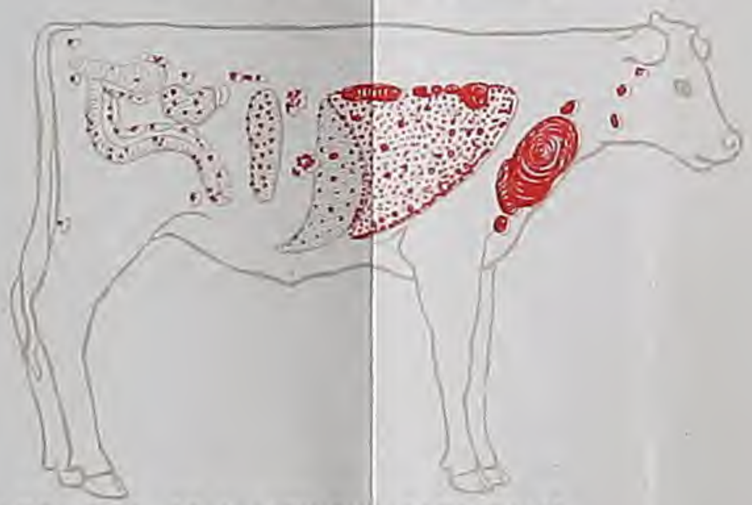
KILLED. 18.9.08. 122 DAYS.

III.

The strain was derived from the original material through G.P. 2929. and was inoculated on January 25, 1909, when it had been 320 days in artificial cultivation.

1523. SUBCUT.

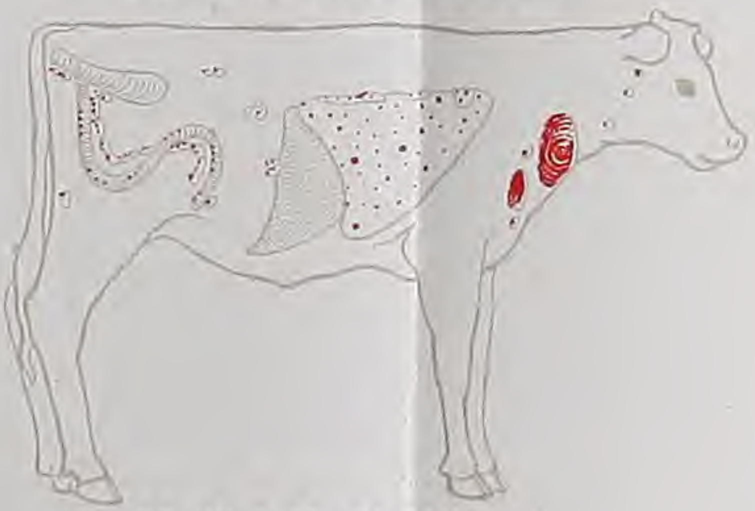
DOSE, 50 MILLIGRAMMES.



KILLED. 8.4.09. (WHEN DYING) 73 DAYS.

1547. SUBCUT.

DOSE, 100 MILLIGRAMMES.



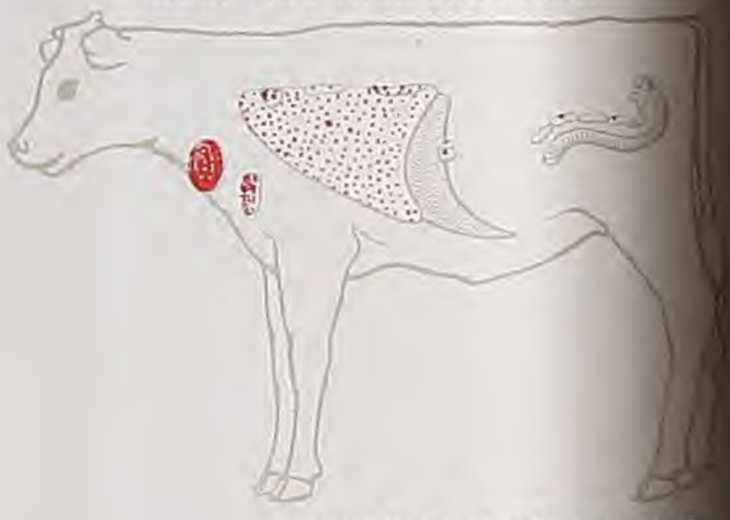
KILLED 30.4.09. 95 DAYS.

II.

The strain was derived from the original material through G.P. 2931. and was inoculated on August 24, 1908. when it had been 148 days in artificial cultivation.

1409. SUBCUT.

DOSE, 100 MILLIGRAMMES.



KILLED. 21.12.08. 119 DAYS.

CULTURE

Derived from the lung of Calf 1419. Inoculated on December 3, 1908. after 76 days artificial cultivation.

1517. SUBCUT.

DOSE, 50 MILLIGRAMMES.

CULTURE

Derived from the liver of Calf 1419. Inoculated on December 18, 1908, after 91 days artificial cultivation.

1511. SUBCUT.

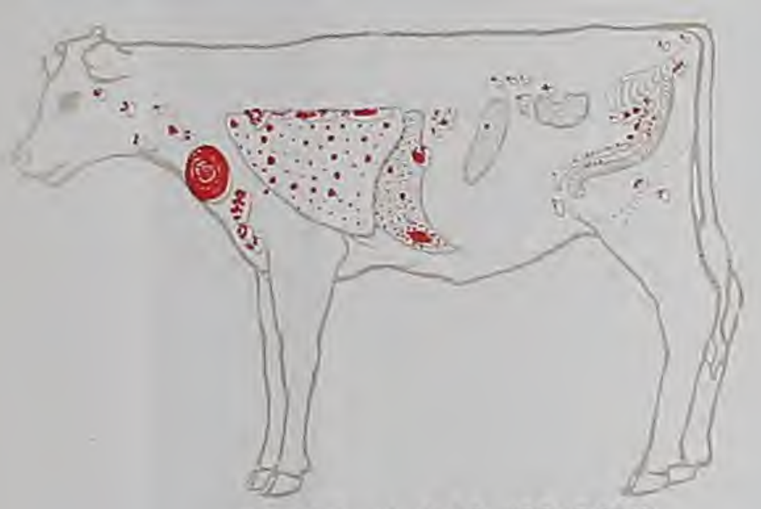
DOSE, 50 MILLIGRAMMES.

CULTURE

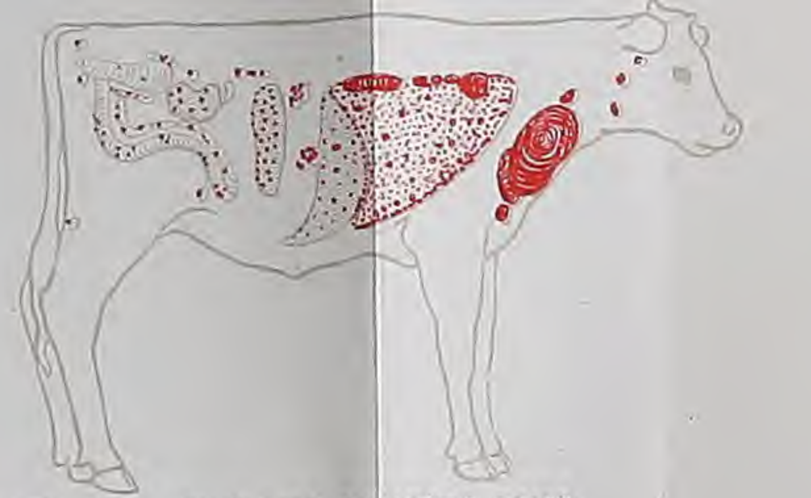
Derived from the mediastinal gland of Calf 1409. Inoculated on February 17, 1909. after 58 days artificial cultivation.

1543. SUBCUT.

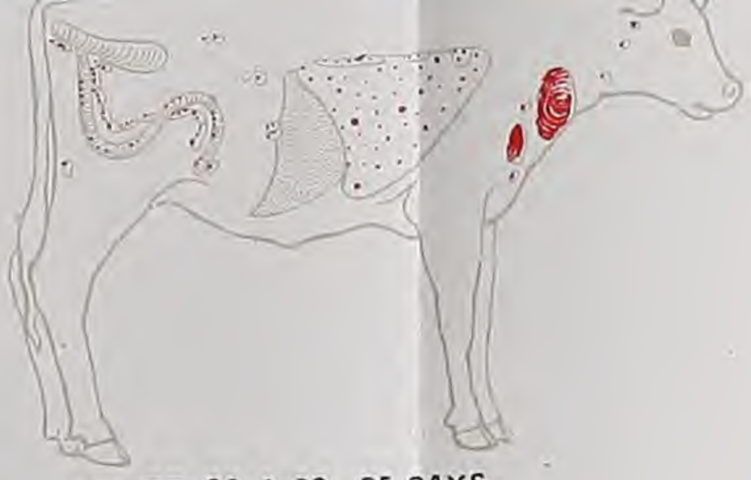
DOSE, 50 MILLIGRAMMES.



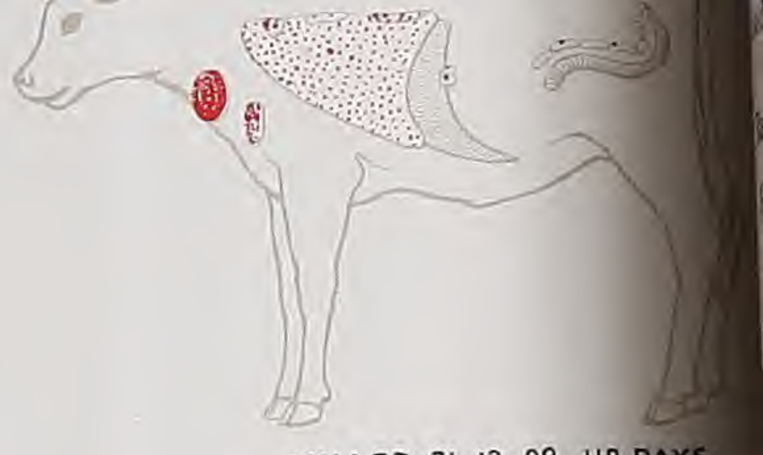
KILLED. 18.9.08. 122 DAYS.



KILLED. 8. 4. 09. (WHEN DYING) 73 DAYS.



KILLED 30.4.09. 95 DAYS.



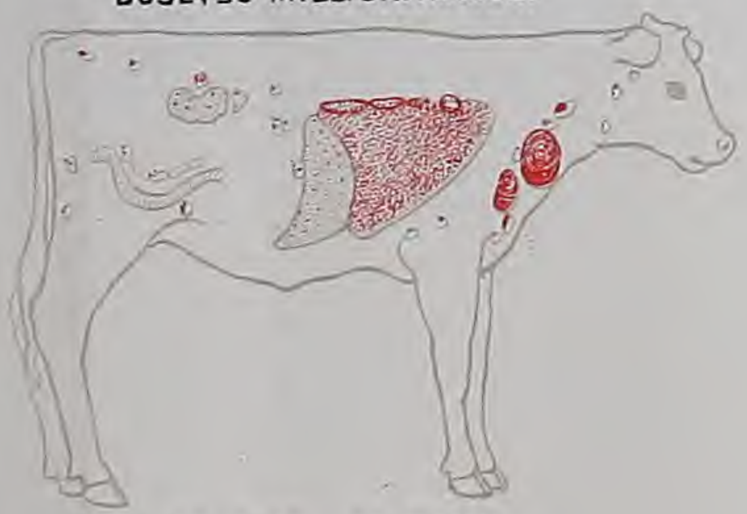
KILLED. 21.12.08. 119 DAYS.

CULTURE

Derived from the lung of Calf 1419. Inoculated on December 3, 1908. after 76 days artificial cultivation.

1517. SUBCUT.

DOSE, 50 MILLIGRAMMES.



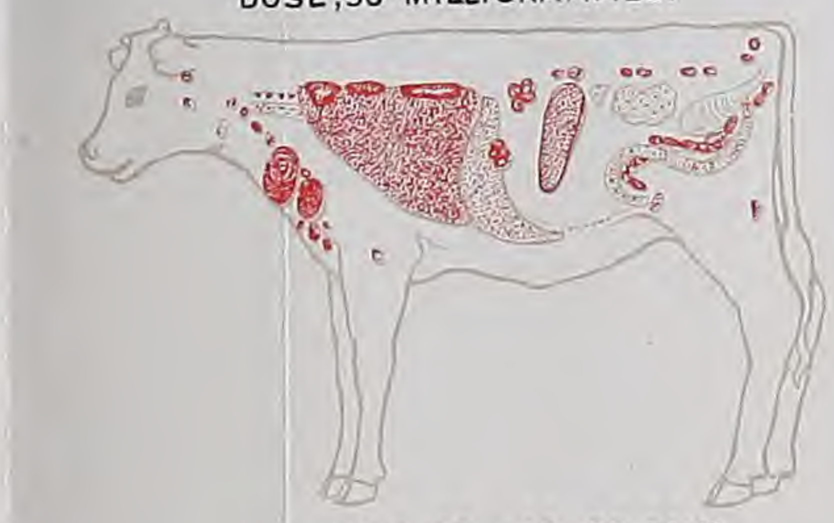
DIED 25.12.08. 22 DAYS

CULTURE

Derived from the liver of Calf 1419. Inoculated on December 18, 1908, after 91 days artificial cultivation.

1511. SUBCUT.

DOSE, 50 MILLIGRAMMES.



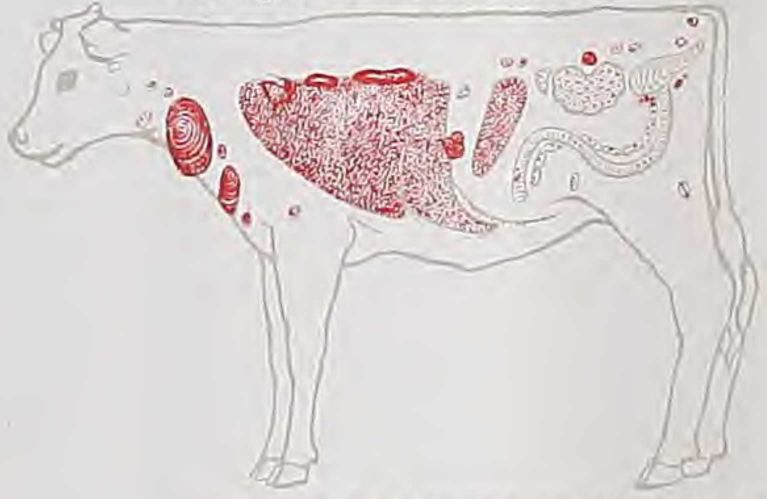
DIED 26.1.09. 39 DAYS.

CULTURE

Derived from the mediastinal gland of Calf 1409. Inoculated on February 17, 1909. after 58 days artificial cultivation.

1543. SUBCUT.

DOSE, 50 MILLIGRAMMES.



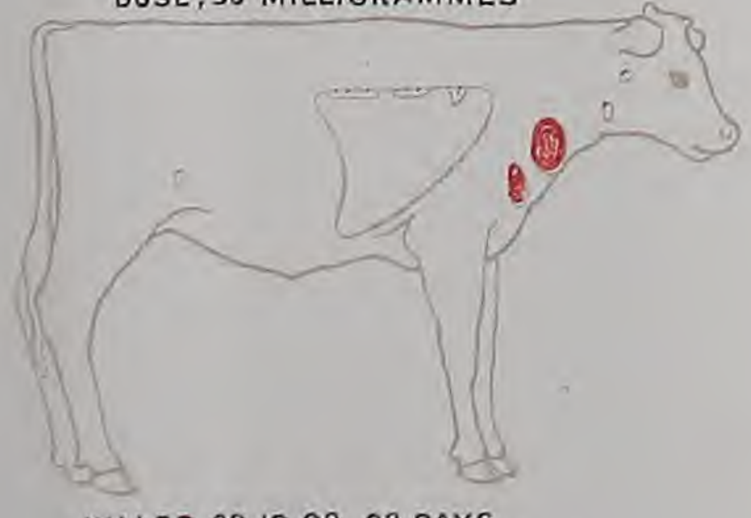
KILLED 23.3.09. (WHEN DYING) 34 DAYS.

VIRUS H. 111. "S.E."

The strain was derived from the original material through G.P. 3009. and was inoculated on July 23, 1908. when it had been 91 days in artificial cultivation.

1429. SUBCUT.

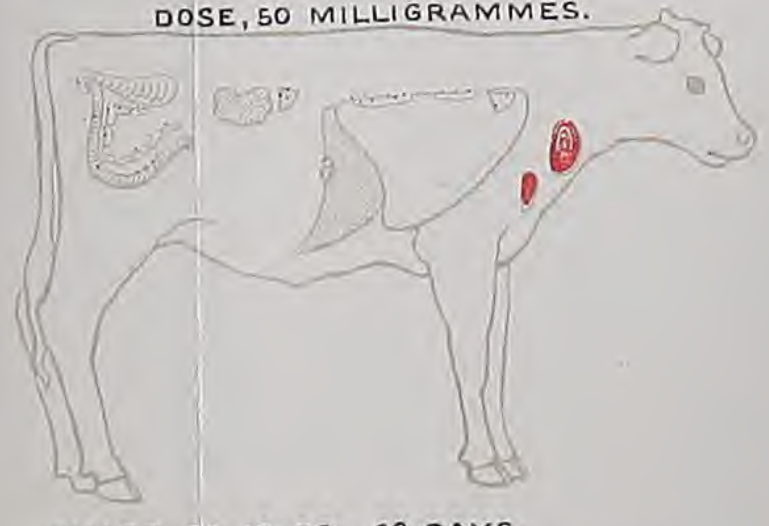
DOSE, 50 MILLIGRAMMES



KILLED. 29.10.08. 98 DAYS.

1459. SUBCUT.

DOSE, 50 MILLIGRAMMES.



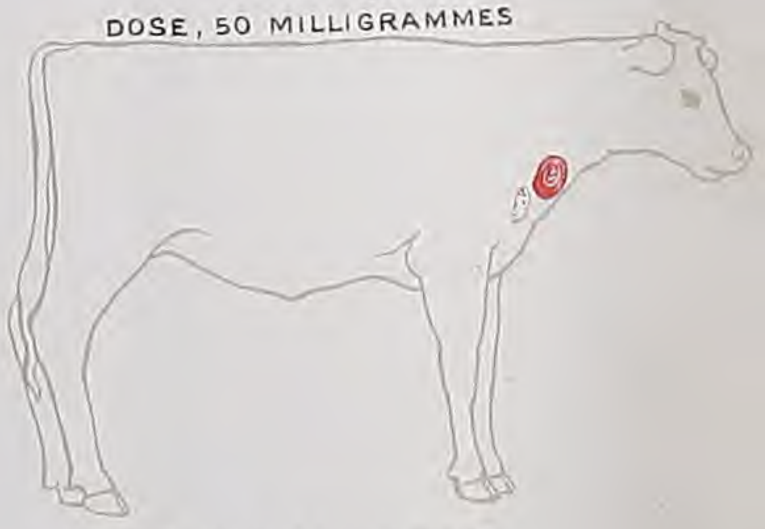
KILLED. 23.10.08. 92 DAYS.
(WHEN DYING OF URETHRAL OBSTRUCTION)

VIRUS H. 91. "H.S."

The strain was derived from the original material through G.P. 2590, and was inoculated on Jan. 20, 1908. when it had been 82 days in artificial cultivation.

1353. SUBCUT.

DOSE, 50 MILLIGRAMMES



KILLED. 18.5.08. 119 DAYS.

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CHART 34.

Inoculations in Calves with four of the Lupus Cultures which grow like Bovine Tubercle Bacilli.

[*See* Appendix to Final Report, Vol. II. : Page 28.]

VIRUS H. 110. "J.B." (*a*) and (*b*). *Lupus of face and trunk; disease of about four years' duration.* The cultures were derived from (*a*) the right flank, and (*b*) the right thigh.

Age of patient : 10 years at the time the (*a*) strain was obtained ; the (*b*) strain was obtained six months later.

The Chart shows the results of inoculation experiments on calves with cultures obtained from both (*a*) and (*b*) strains of the original material.

References.—Final Report : Pages 16 to 19.

Appendix to Final Report, Vol. II. : Pages 64, 65, 449, and 460.

VIRUS H. 100. "R.S." *Lupus of face, neck, arm, and hand ; duration of disease, 17 years.* The culture was derived from the arm.

Age of patient : 37 years.

The Chart shows the results of inoculation experiments with cultures on calves made to determine (i) the original virulence of the virus, and (ii) the virulence of the virus after residence in the body of the calf.

References.—Final Report : Pages 16 to 19, 30 and 31.

Appendix to Final Report, Vol. II. : Pages 52, 134, and 319.

VIRUS H. 111. "S.E." *Lupus of neck ; duration of disease, 10 years.*

Age of patient : 17 years.

The Chart shows the results of inoculation experiments on calves with a culture obtained from the original material.

References.—Final Report : Pages 16 to 19.

Appendix to Final Report, Vol. II. : Pages 66 and 463.

VIRUS H. 91. "H.S." *Lupus of chin ; duration of disease, 3½ years.*

Age of patient : 9½ years.

The Chart shows the result of an inoculation experiment on a calf with a culture of the original material.

References.—Final Report : Pages 16 to 19.

Appendix to Final Report, Vol. II. : Pages 49 and 297.

TEST INOCULATIONS IN CALVES AND PASSAGE EXPERIMENTS WITH TWO OF THE LUPUS CULTU WHICH GROW LIKE BOVINE TUBERCLE BACILLI.

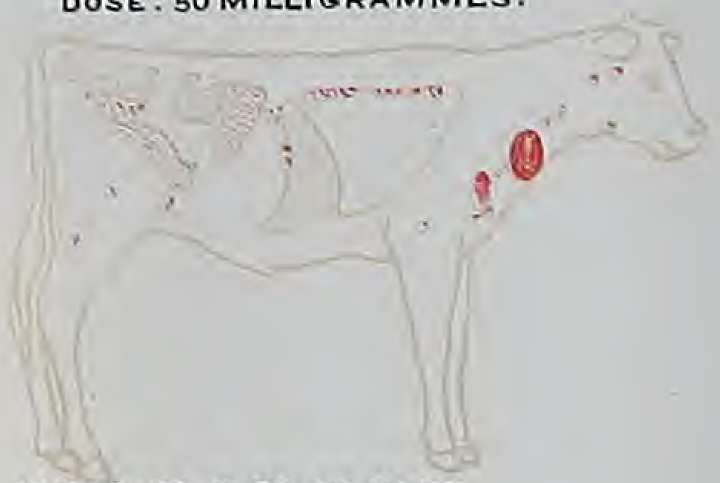
VIRUS H 85. "H.B".

I.

The strain was derived from the original material through G.P. 2363, and was inoculated on Aug. 20, 1907. when it had been 83 days in artificial cultivation.

1289. SUBCUT.

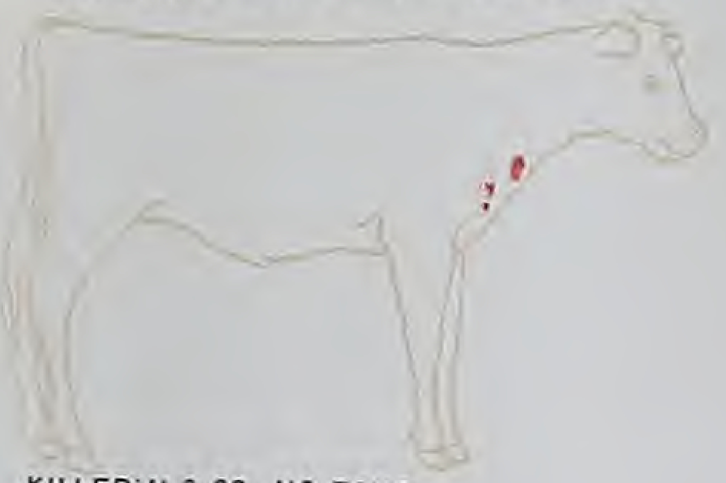
DOSE: 50 MILLIGRAMMES.



KILLED: 22.11.07. 94 DAYS.

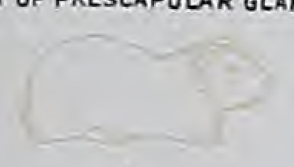
1319. SUBCUT.

DOSE: 20 C.C. OF EMULSION OF PRESCAPULAR GLAND CONTAINING A MODERATE NUMBER OF TUBERCLE BACILLI.



KILLED: 11.3.08. 110 DAYS.

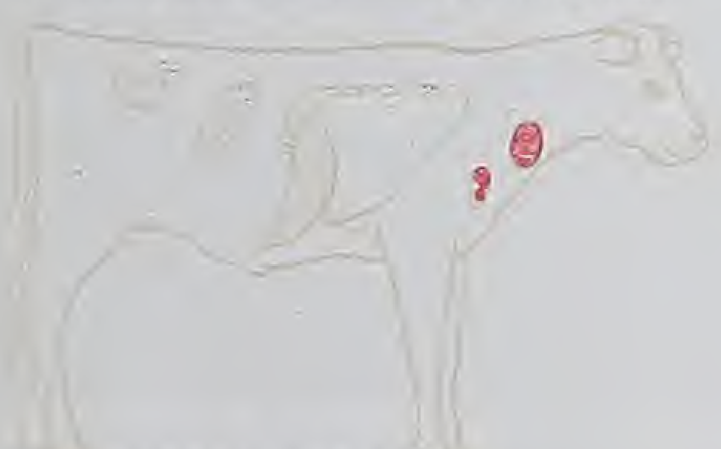
EMULSION OF PRESCAPULAR GLAND INTO:—



[8 4 .08.]

1397. SUBCUT.

DOSE: 20 C.C. OF EMULSION OF G.P.'s TISSUES CONTAINING NOT VERY NUMEROUS TUBERCLE BACILLI.



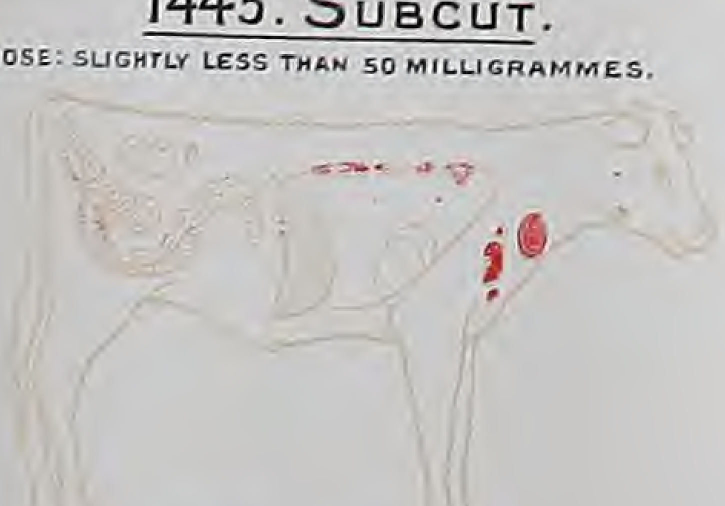
KILLED: 30.7.08. 113 DAYS.

CULTURE.

Derived from the Thoracic gland of Calf 1397. Inoculated on Oct. 3. 1908. after 65 days artificial cultivation.

1445. SUBCUT.

DOSE: SLIGHTLY LESS THAN 50 MILLIGRAMMES.



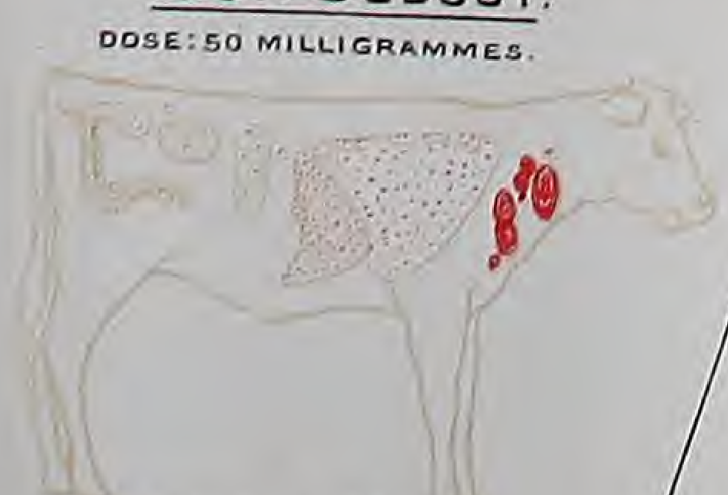
KILLED: 6.1.09. 95 DAYS.

CULTURE.

Derived from the Mediastinal gland of Calf 1445. Inoculated on Feb. 17. 1909. after 42 days artificial cultivation

1531. SUBCUT.

DOSE: 50 MILLIGRAMMES.



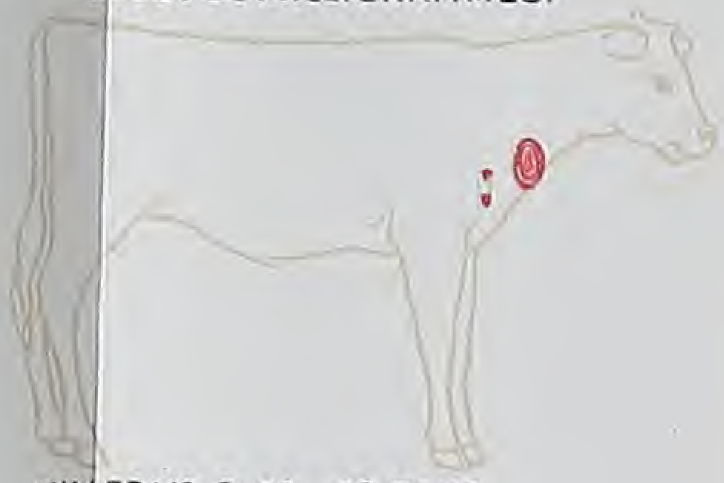
KILLED: 12.5.09. 84 DAYS.

II.

The strain was derived from the original material through G.P. 2365. and was inoculated on Dec. 13, 1907. when it had been 189 days in artificial cultivation.

1331. SUBCUT.

DOSE: 96 MILLIGRAMMES.



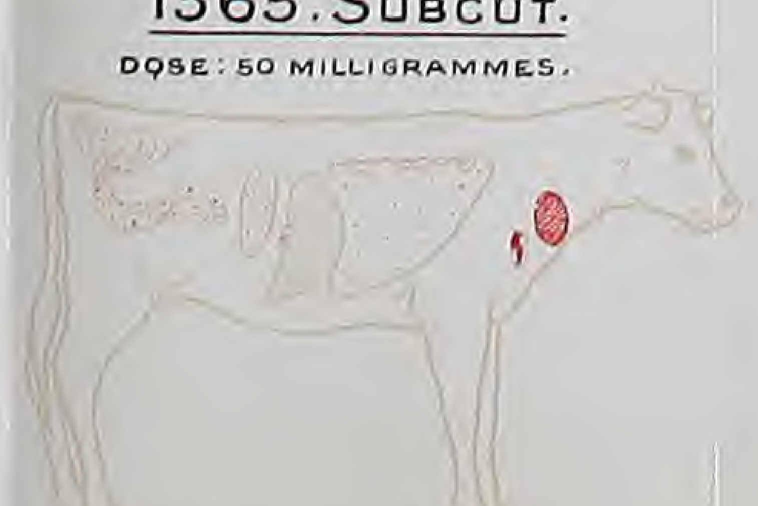
KILLED: 12.3.08. 90 DAYS.

CULTURE.

Derived from the liver of Calf 1531. Inoculated on June 28, 1909, after 47 days artificial cultivation.

1565. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 5.10.09. 99 DAYS.

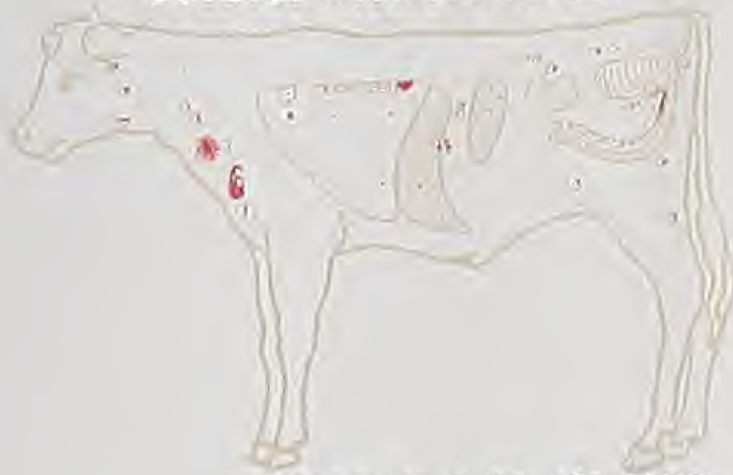
VIRUS H 108. "H.R".

I.

The strain was derived from the original material through G.P. 2957. and was inoculated on May 19, 1908. when it had been 57 days in artificial cultivation

1417. SUBCUT.

DOSE: 50 MILLIGRAMMES.



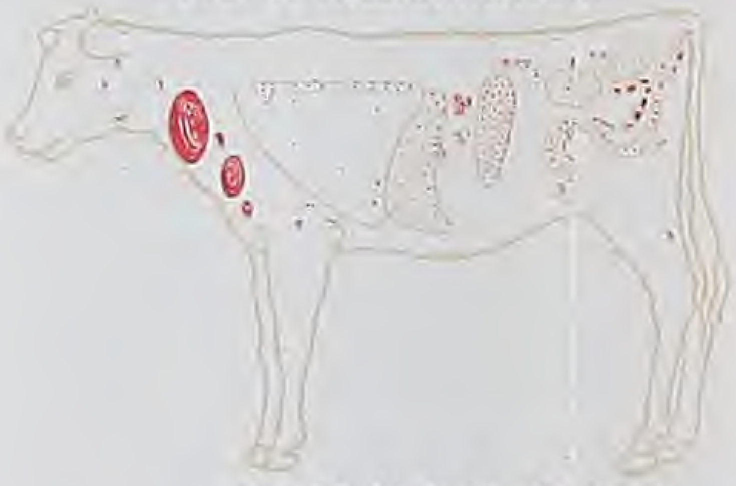
KILLED: 21.9.08. 125 DAYS.

CULTURE.

Derived from the Mediastinal gland of Calf 1417. Inoculated on Jan. 19. 1909. after 120 days artificial cultivation.

1533. SUBCUT.

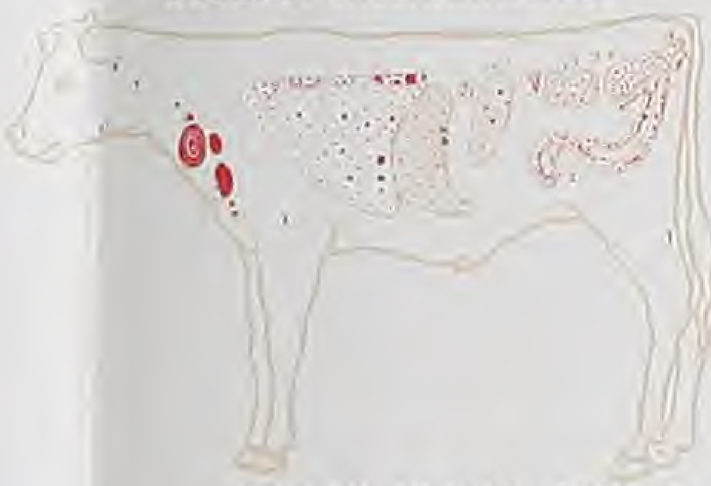
DOSE: 50 MILLIGRAMMES.



KILLED: 27.4.09. 98 DAYS.

1537. SUBCUT.

DOSE: 50 MILLIGRAMMES.



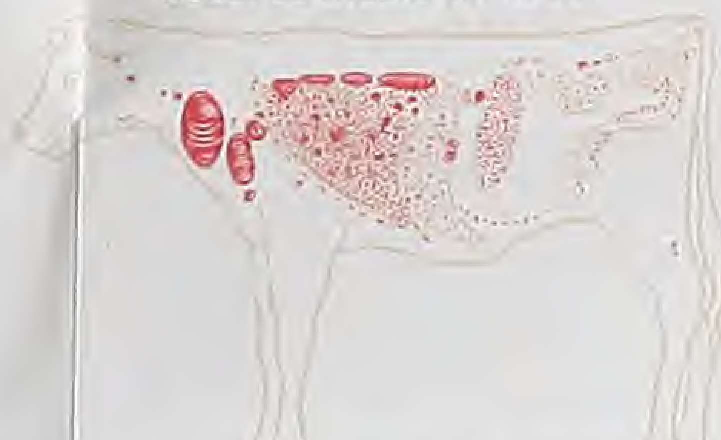
KILLED: 28.4.09. 99 DAYS.

CULTURE.

Derived from the Mediastinal gland of Calf 1537. Inoculated on Oct. 6. 1909. after 161 days artificial cultivation.

1605. SUBCUT.

DOSE: 50 MILLIGRAMMES.



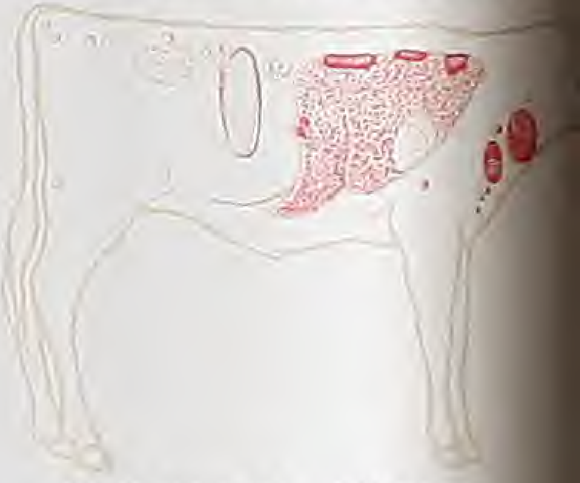
DIED: 29.11.09. 54 DAYS.

CULTURE.

Derived from the Lung of Cal
Inoculated on Jan. 5, 1909. a
106 days artificial cultivat

1539. SUBCUT.

DOSE: 50 MILLIGRAMMES



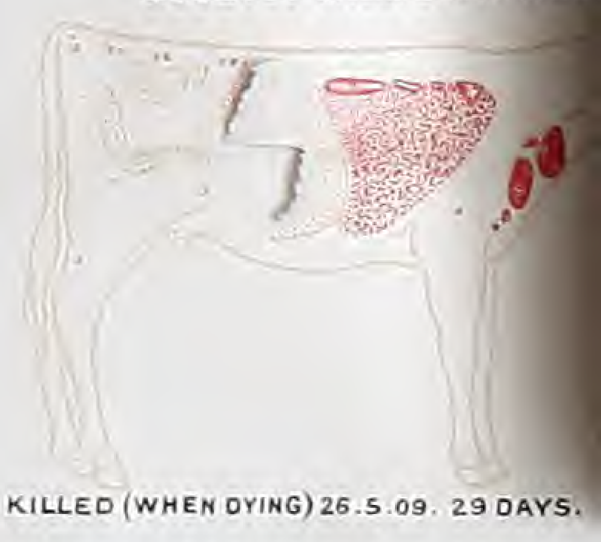
DIED: 21.1.09. 16½ DAYS.

CULTURE.

Derived from the Bronchial gl
Calf 1539. Inoculated on April 2
after 96 days artificial cultivat

1509. SUBCUT.

DOSE: 50 MILLIGRAMMES.



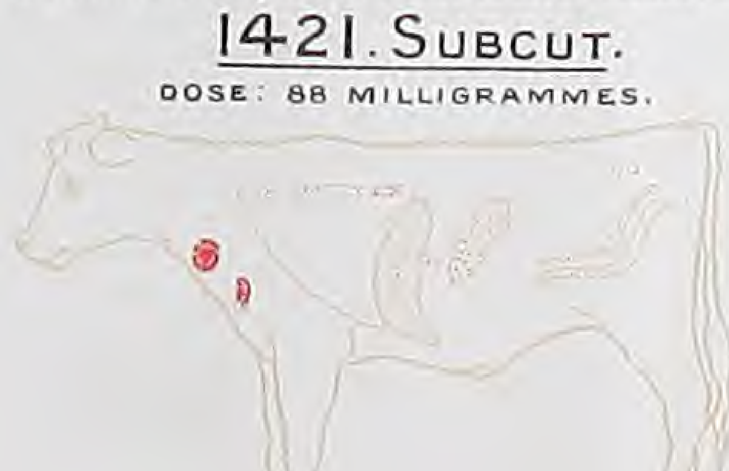
KILLED (WHEN DYING) 26.5.09. 29 DAYS.

II.

The strain was derived from the original material through G.P. 2957. and was inoculated on Sep. 9. 1908. when it had been 170 days in artificial cultivation.

1421. SUBCUT.

DOSE: 88 MILLIGRAMMES.



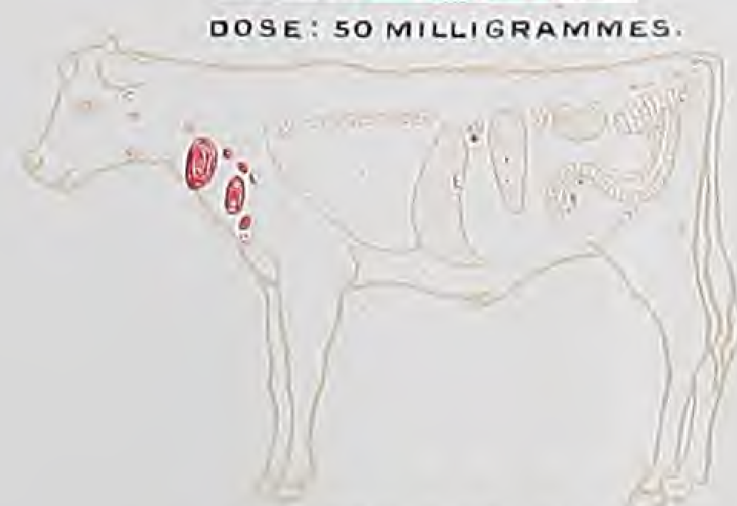
KILLED: 19.12.08. 101 DAYS.

CULTURE.

Derived from the Spleen of Calf 1421. Inoculated on Feb. 26, 1909. after 69 days artificial cultivation.

1553. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 2.6.09. 96 DAYS.

CHART 35.

"Inoculations in Calves and Passage Experiments with two of the Lupus Cultures which grow like Bovine Tubercle Bacilli."

[*See* Appendix to Final Report, Vol. II.. page 28.]

VIRUS H. 105. "G.S." *Lupus of palate, nose, upper lip, and cheek; duration of disease, 1 year.* The culture was derived from nodules excised from the nose and the cheek.

Age of patient: 15 years.

The Chart shows the experiments on calves made to determine (1) the original virulence of the virus, and (2) the virulence after residence in the bodies of successive calves.

References.—Final Report: Pages 16 to 19, 30 and 31.

Appendix to Final Report, Vol. II.: Pages 58, 140, and 361

VIRUS H. 107. "H.H." *Lupus of face, neck, and arms; duration of disease, 8 years.* The culture was derived from the right elbow.

Age of patient: 17 years.

The Chart shows experiments on calves made to determine (i) the original virulence of the virus and (ii) the virulence after residence in the bodies of successive calves.

References.—Final Report: Pages 16 to 19, 30 and 31.

Appendix to Final Report, Vol. II.: Pages 60, 144, and 395.

VIRUS H. 53. "D.H."

LUPUS.

CULTURE INOCULATIONS.

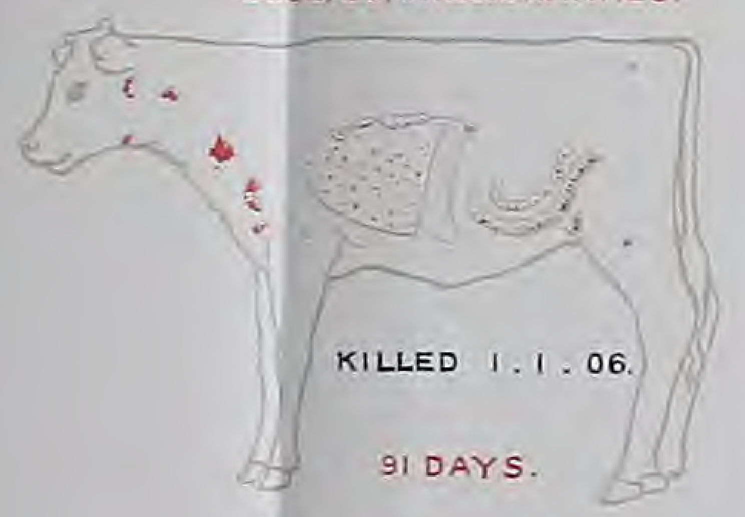
STRAIN DERIVED FROM HUMAN SKIN, THROUGH G.P. 1482.

OCTOBER 2ND 1905.

TOTAL DURATION OF ARTIFICIAL CULTIVATION 8½ MONTHS

905. SUBCUT.

DOSE, 50, MILLIGRAMMES.



KILLED 1. 1. 06.

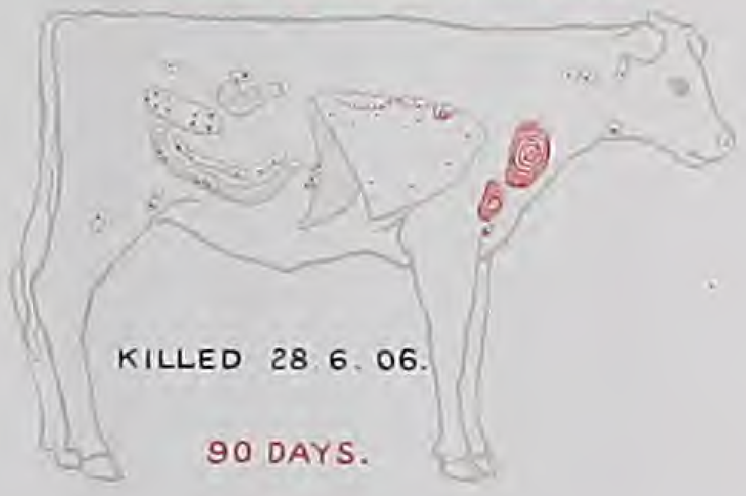
91 DAYS.

MARCH 30TH 1906.

TOTAL DURATION OF ARTIFICIAL CULTIVATION, 14½ MONTHS.

977. SUBCUT.

DOSE, 50, MILLIGRAMMES.

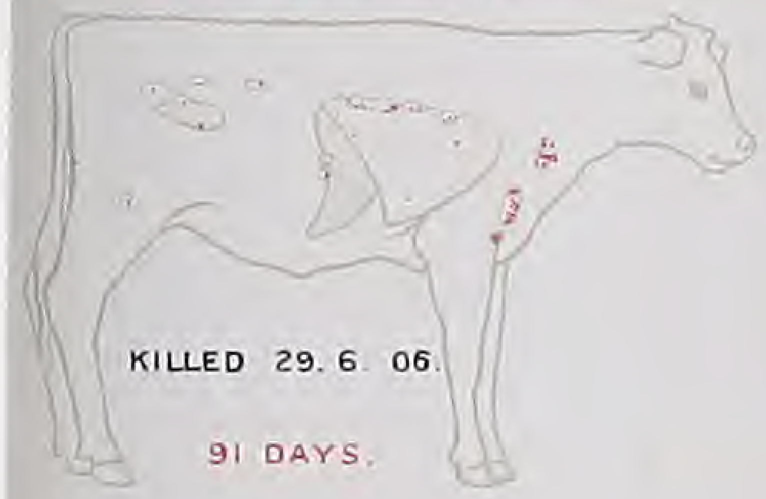


KILLED 28. 6. 06.

90 DAYS.

1001. SUBCUT.

DOSE, 50 MILLIGRAMMES.



KILLED 29. 6. 06.

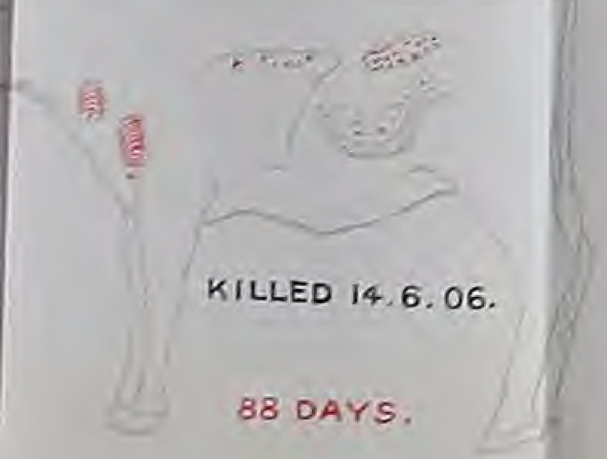
91 DAYS.

MARCH, 18TH 1906.

STRAIN DERIVED FROM CALF 905, DIRECT.
TOTAL DURATION OF ARTIFICIAL CULTIVATION, 2½ MONTHS.

973. SUBCUT.

DOSE, 10, MILLIGRAMMES.

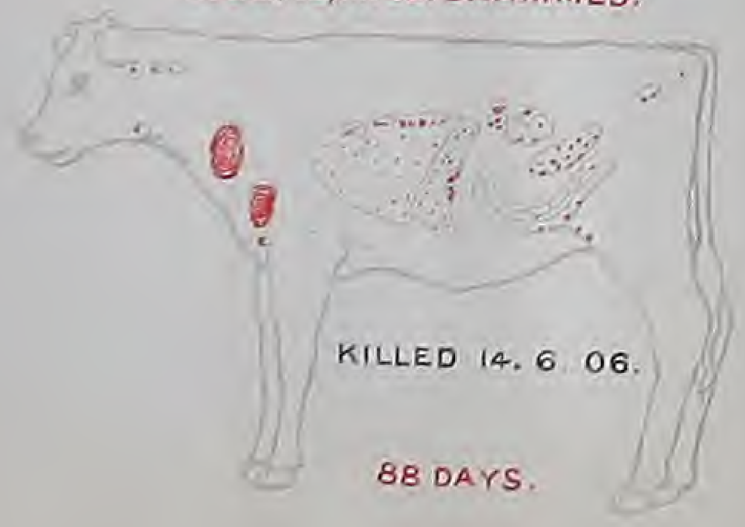


KILLED 14. 6. 06.

88 DAYS.

975, SUBCUT.

DOSE, 10, MILLIGRAMMES.

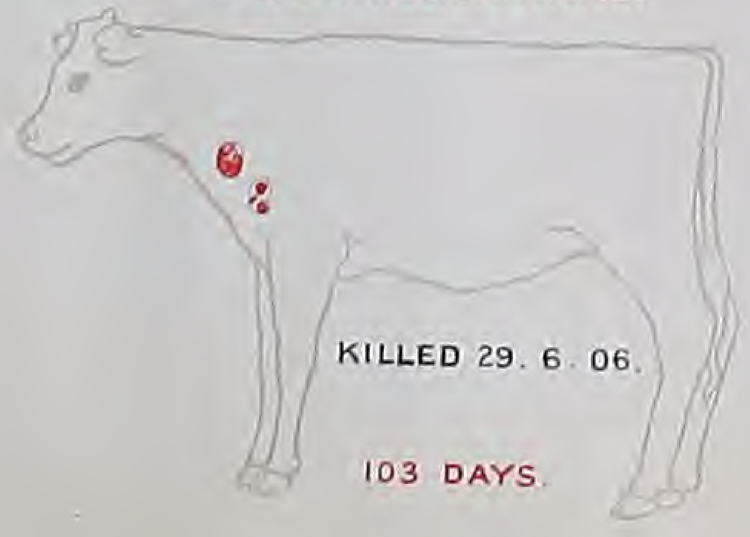


KILLED 14. 6. 06.

88 DAYS.

979. SUBCUT.

DOSE, 50, MILLIGRAMMES.

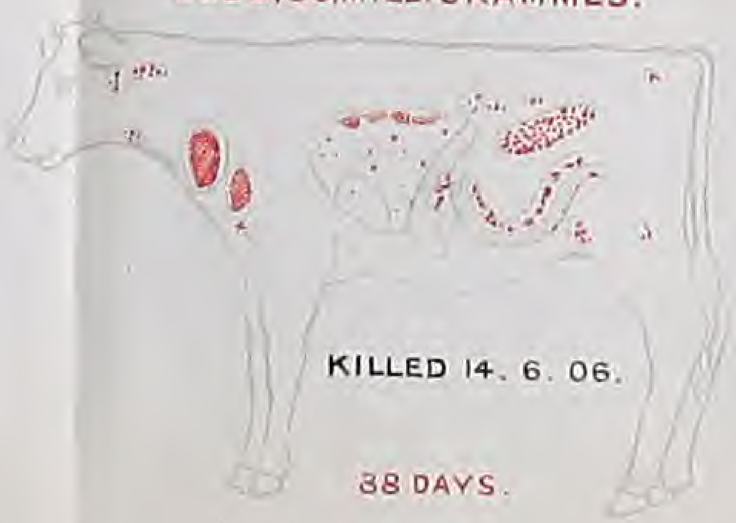


KILLED 29. 6. 06.

103 DAYS.

993. SUBCUT.

DOSE, 50, MILLIGRAMMES.



KILLED 14. 6. 06.

88 DAYS.

[illegible] Ltd, Litho. London.

CHART 36.

Test Inoculations in Calves and Passage Experiments with two of the Lupus Cultures which grow like Bovine Tubercle Bacilli.

[*See* Appendix to Final Report, Vol. II., page 28.]

VIRUS H. 85. "H.B." *Lupus of the nose; duration of disease, about 3 months.* The culture was derived from scrapings from the nose.

Age of patient: 5 years.

The Chart shows the experiments on calves made to determine (i) the original virulence of the virus, and (ii) the virulence of the virus after residence in the bodies of successive calves.

[For experiments with Virus H. 85. "H.B." on Pigs and Goats *see* Chart 39.]

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 46, 147, and 265.

VIRUS H. 108. "H.R." *Lupus of the face, arm, and buttock; duration of disease, 5 years.* The culture was derived from the arm.

Age of patient: $8\frac{1}{2}$ years.

The Chart shows the experiments on calves made to determine (i) the original virulence of the virus, and (ii) the virulence after residence in the bodies of successive calves.

References.—Final Report: Pages 16 to 19, 30 and 31.

Appendix to Final Report, Vol. II.: Pages 62, 137, and 421.

TEST INOCULATIONS IN CALVES AND PASSAGE EXPERIMENTS WITH THE LUPUS VIRUS H 53. "D.H." *(a)* AND *(b)*. THE CULTURES FROM WHICH GROW LIKE BOVINE TUBERCLE BACILLI.

THE ORIGINAL MATERIAL OF H 53. D.H. *(b)* WAS TAKEN FROM THE PATIENT 3 7/12 YEARS AFTER THAT OF H 53. D.H. *(a)*.

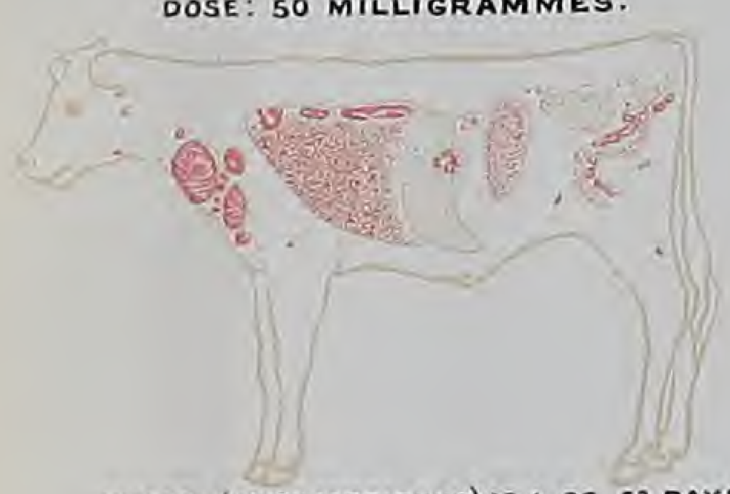
VIRUS H 53. D.H. *(b)*.

I.

The strain was derived from the original material through G.P. 3215, and was inoculated on November 14, 1908. when it had been 63 days in artificial cultivation

1507. SUBCUT.

DOSE: 50 MILLIGRAMMES.



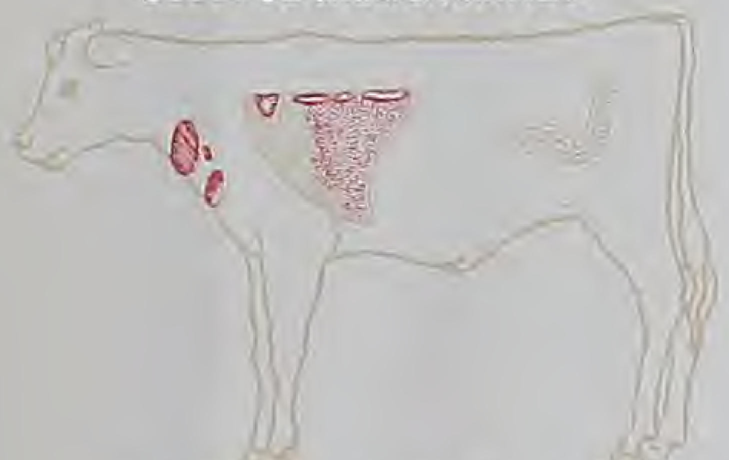
KILLED (WHEN MORIBUND) 16.1.09. 63 DAYS.

CULTURE.

Derived from the portal gland of Calf 1507. Inoculated on March 2, 1909. after 45 days artificial cultivation.

1555. SUBCUT

DOSE: 50 MILLIGRAMMES.



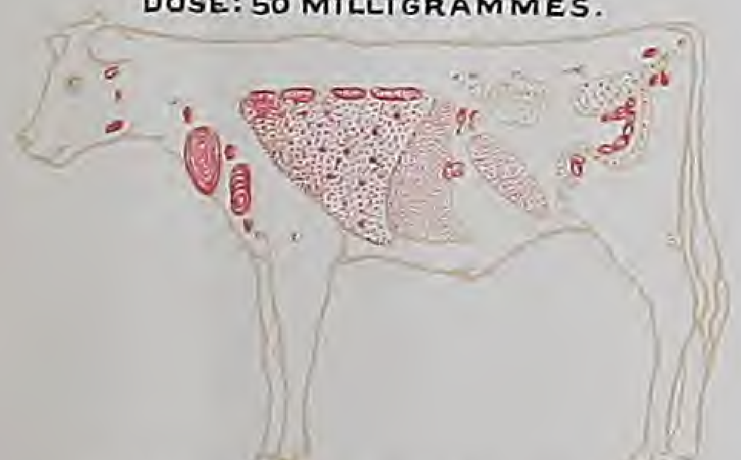
DIED (OF PNEUMONIA NOT TUBERCULOUS) 28.3.09. 26 DAYS.

CULTURE.

Derived from the small mediastinal gland of Calf 1555. Inoculated on May 22, 1909. after 55 days artificial cultivation.

1563. SUBCUT.

DOSE: 50 MILLIGRAMMES.



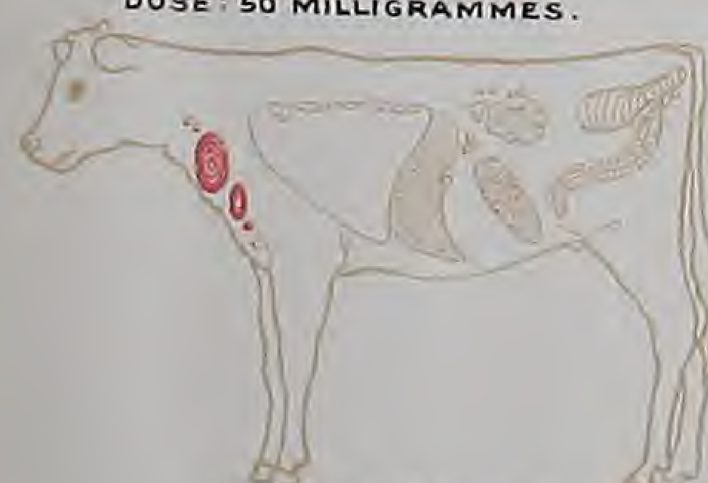
KILLED (WHEN VERY ILL) 17.8.09. 87 DAYS.

CULTURE.

Derived from the popliteal gland of Calf 1563. Inoculated on October 6. 1909. after 50 days artificial cultivation.

1599. SUBCUT.

DOSE: 50 MILLIGRAMMES.

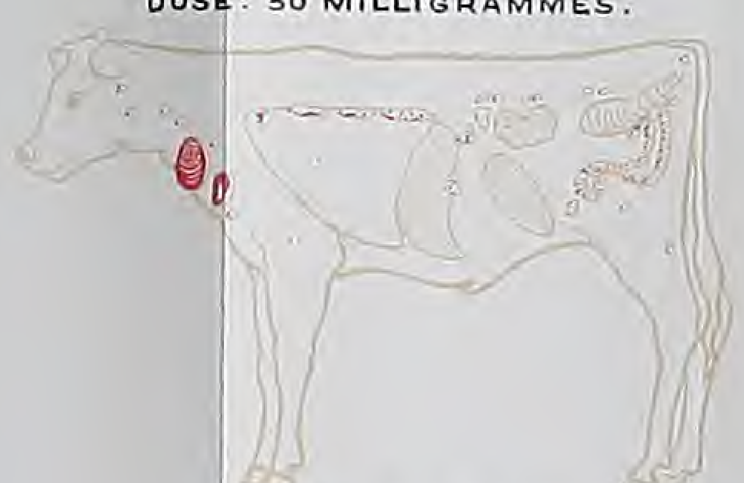


KILLED 19.1.10. 105 DAYS.

Inoculated on April 29. 1909, after 103 days artificial cultivation.

1559. SUBCUT.

DOSE: 50 MILLIGRAMMES.



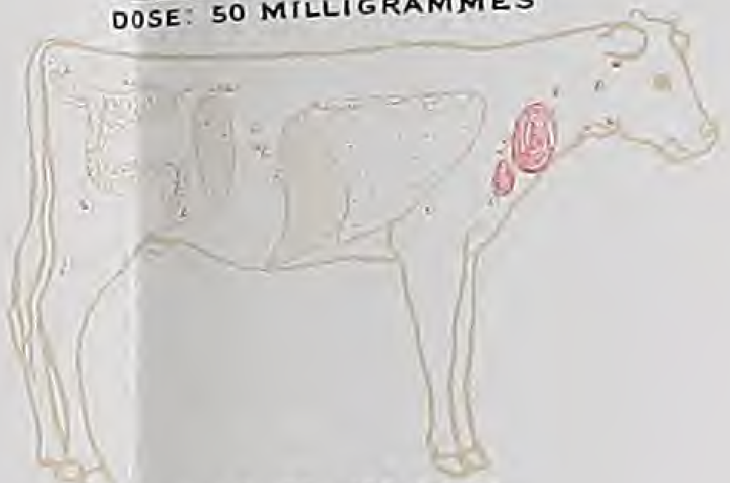
KILLED: 18.8.09. 111 DAYS.

II.

The strain was derived from the original material through G.P. 3216. and was inoculated on January 21. 1909. when it had been 120 days in artificial cultivation.

1535. SUBCUT.

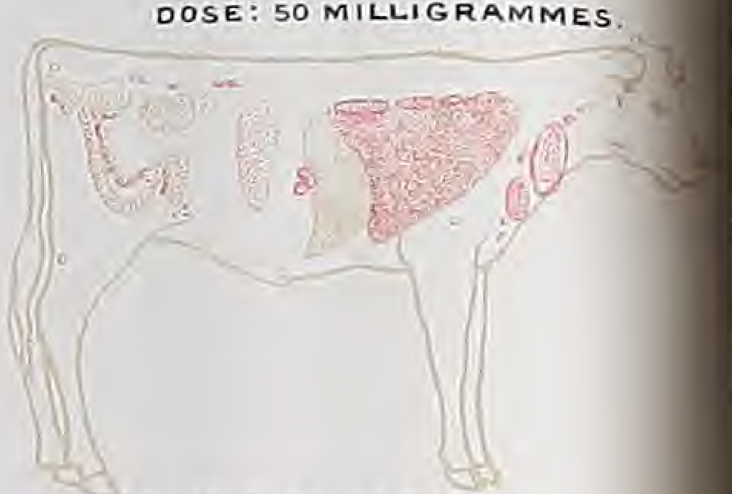
DOSE: 50 MILLIGRAMMES



KILLED: 4. 5. 09. 103 DAYS

1545. SUBCUT.

DOSE: 50 MILLIGRAMMES.



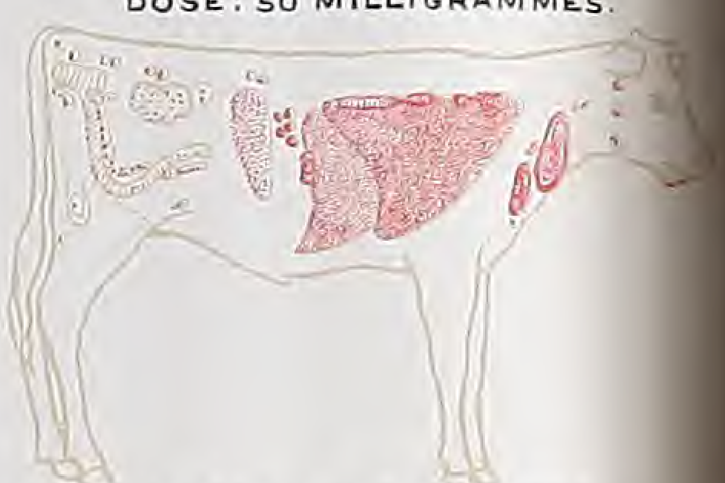
DIED: 14. 3. 09. 52 DAYS.

CULTURE.

Derived from the portal gland of Calf 1545. Inoculated on May 6. 1909. after 53 days artificial cultivation.

1561. SUBCUT.

DOSE: 50 MILLIGRAMMES.



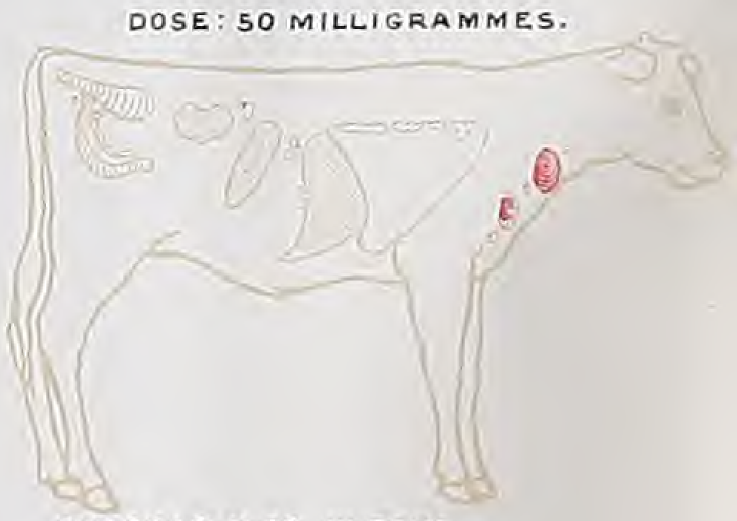
KILLED (WHEN DYING) 14. 6. 09. 39 DAYS.

CULTURE.

Derived from a mediastinal gland of Ca 1561. Inoculated on July 15, 1909. after 31 days artificial cultivation.

1571. SUBCUT.

DOSE: 50 MILLIGRAMMES.



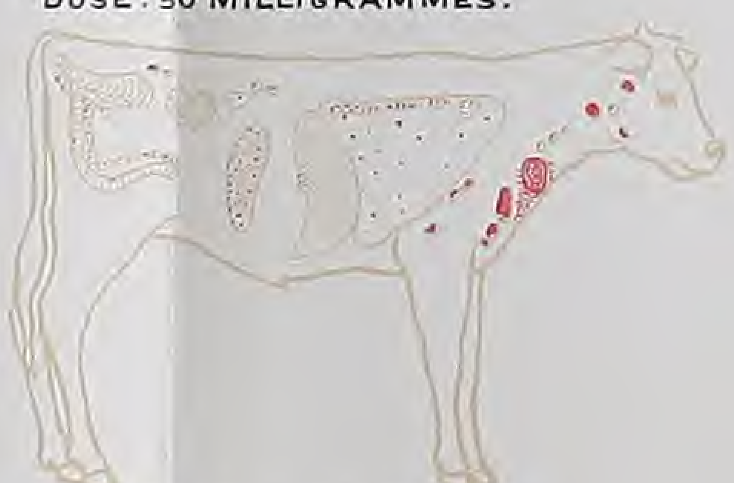
KILLED: 3. 11. 09. 111 DAYS.

VIRUS H 53. "D.H." *(a)*

The strain was derived from the original material through G.P. 1482. and was inoculated on January 21. 1907. when it had been 688 days in artificial cultivation.

1155. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 6. 6. 07. 136 DAYS.

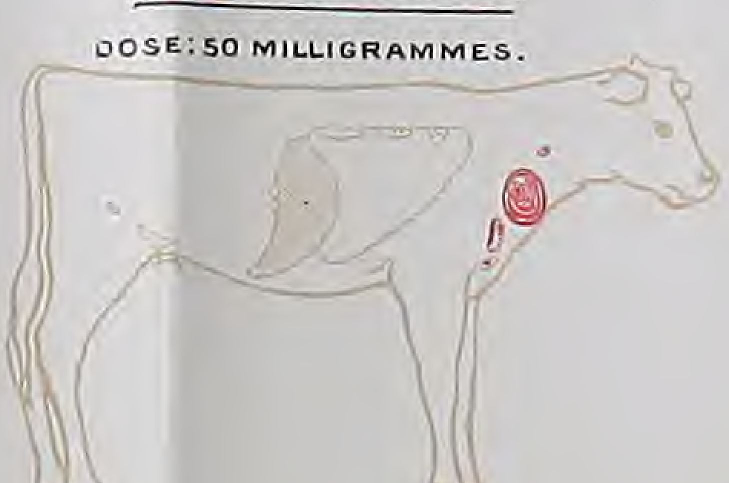
The virus was passed in tissue emulsions through a series of three more Calves in succession and the culture was recovered from the last of the series (Calf 1367): the total duration of residence in the body of the Calf was 506 days.

CULTURE.

Derived from a thoracic gland of Calf 1367. Inoculated October 22. 1908. after 134 days artificial cultivation.

1461. SUBCUT.

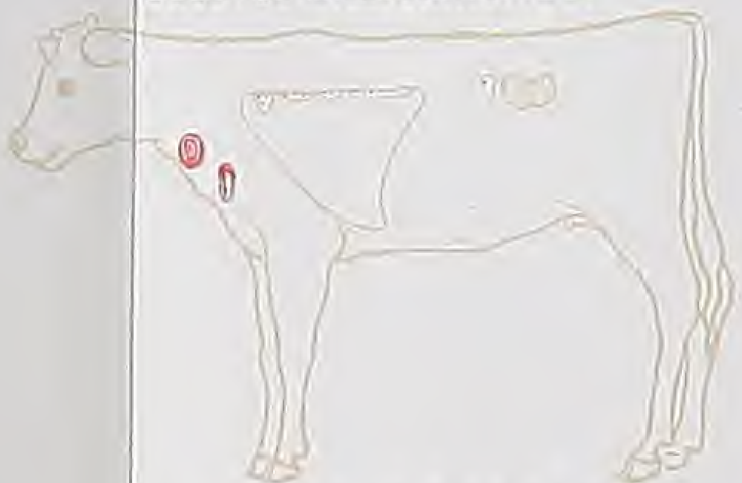
DOSE: 50 MILLIGRAMMES.



KILLED: 10. 2. 09. 111 DAYS.

1135. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 10. 4. 07. 79 DAYS.

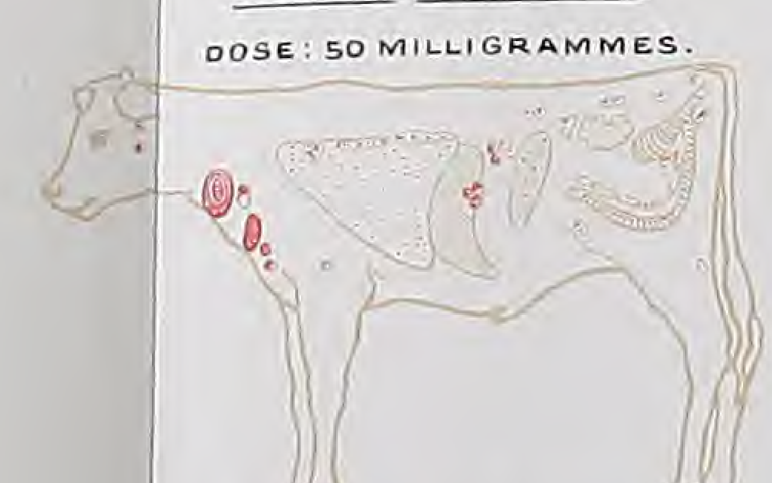
The virus was passed in tissue emulsions through a series of three more Calves in succession and the culture was recovered from the last of the series (Calf 1341): the total duration of residence in the body of the Calf was 429 days.

CULTURE.

Derived from a suprarenal body of Calf 1341. Inoculated on September 3. 1908. after 98 days artificial cultivation.

1477. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 8. 12. 08. 96 DAYS.

CHART 37.

VIRUS H. 53. " D.H." Lupus.

H. 53. " D.H." was the only lupus virus which had been obtained and partly investigated at the time of the issue of the 2nd Interim Report. The patient from which it was obtained was a girl aged 15 years who had suffered from the disease since childhood : the lupus was situated on the hip. Cultures were isolated through the guinea-pig from scrapings of the diseased area.

The virus was provisionally placed by the Commission in Group III. in that report, pending further investigation ; since its virulence appeared to be intermediate between that of Group I. and that of Group II.

Chart 37 shows the nature and extent of the first investigation ; inoculation experiments were carried out on calves to determine (i) the original virulence of the virus and (ii) whether any alteration in the virulence of the virus for the calf had occurred as a result of residence in the body of the calf. It will be seen that no alteration in virulence was demonstrated.

References.—2nd Interim Report : Page 30, para. 52 ; page 34, paras. 60 to 65 ; page 89.

Appendix to 2nd Interim Report, Vol. II. : Pages 16 and 882 to 897.

Further investigations were carried out by Dr. Griffith subsequent to the issue of the 2nd Interim Report, not only with the original culture of Virus H. 53. " D.H." (now called H. 53. " D.H." (*a*)) but also with material obtained from the same patient 3 years and 7 months later (Virus H. 53. " D.H." (*b*)). (*See* Chart 37A for details.)

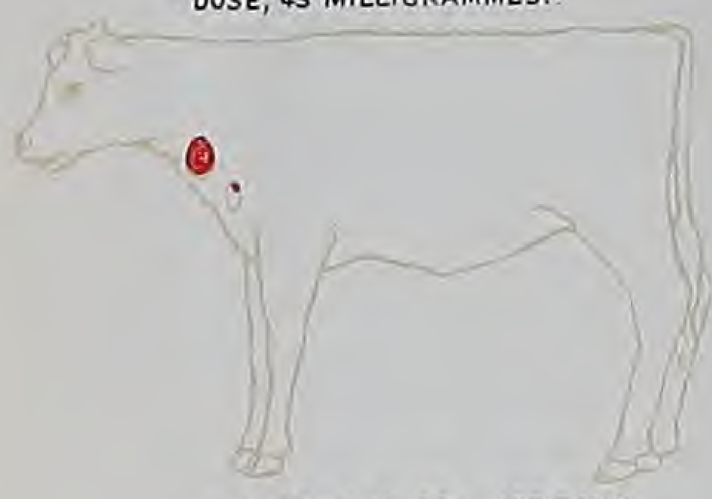
CULTURE INOCULATIONS IN CALVES OF THE ELEVEN EASY-GROWING LUPUS VIRUSES.

VIRUS H. 102. “N.H.”

The strain was derived from the original material through G.P. 2934 and was inoculated on May. 20, 1908. when it had been 62 days in artificial cultivation.

1423. SUBCUT.

DOSE, 43 MILLIGRAMMES.



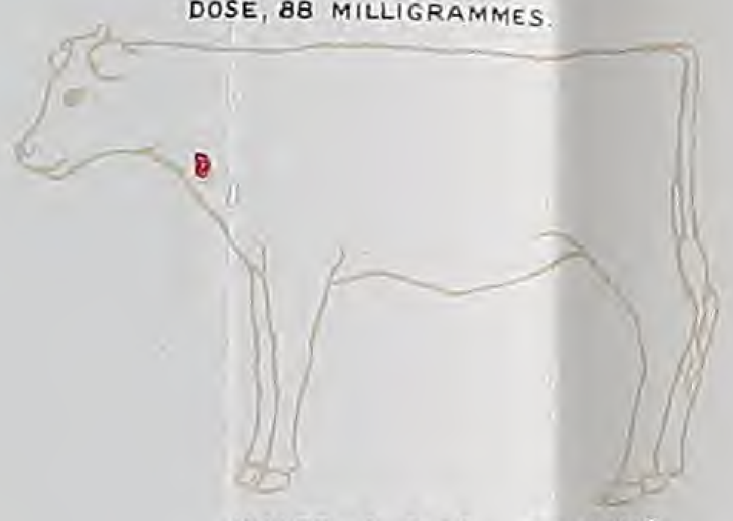
KILLED. 4. 9. 08. 107 DAYS.

VIRUS H. 103. “N.S.”

The strain was derived from the original material through G.P. 2940 and was inoculated on May 20 1908. when it had been 58 days in artificial cultivation.

1457. SUBCUT.

DOSE, 88 MILLIGRAMMES.



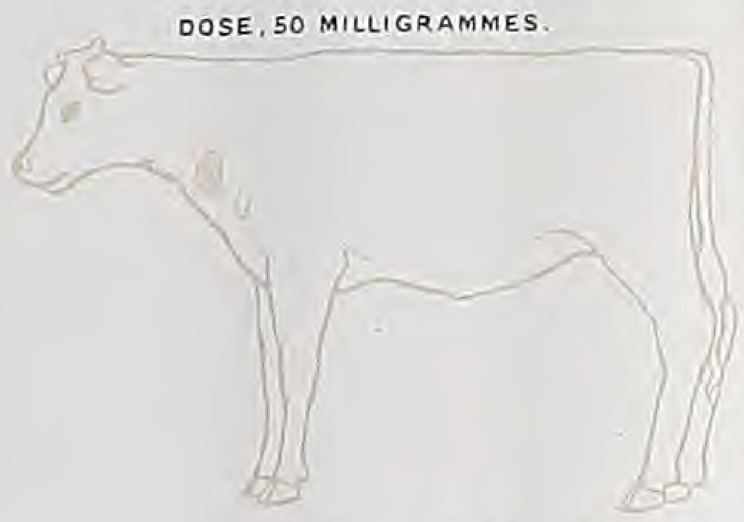
KILLED 4. 9. 08. 107 DAYS.

VIRUS H. 106. “K.R.”

The strain was derived from the original material through G.P. 2952 and was inoculated on April 28, 1908. when it had been 38 days in artificial cultivation.

1403. SUBCUT.

DOSE, 50 MILLIGRAMMES.



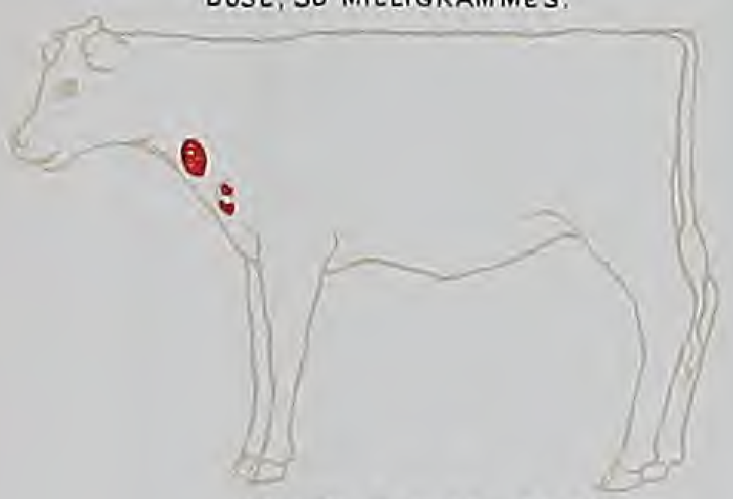
KILLED. 18. 8. 08. 112 DAYS.

VIRUS H. 84. “M.S.”

The strain was derived from the original material through G.P. 2277 and was inoculated on August 7, 1907. when it had been 102 days in artificial cultivation.

1273. SUBCUT.

DOSE, 50 MILLIGRAMMES.



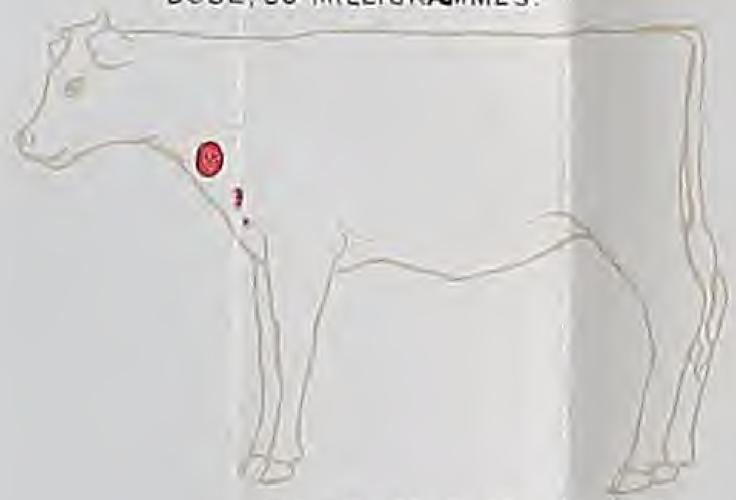
KILLED 4. 11. 07. 89 DAYS.

VIRUS H. 101. “E.G.”

The strain was derived from the original material through G.P. 2932 and was inoculated on May 22 1908. when it had been 56 days in artificial cultivation.

1447. SUBCUT.

DOSE, 50 MILLIGRAMMES.



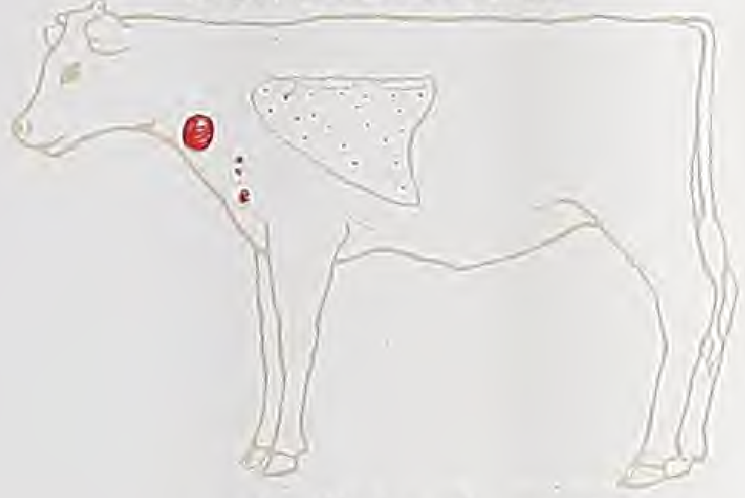
KILLED 22. 9. 08. 123 DAYS.

VIRUS H. 114. “A.U.”

The strain was derived from the original material and was inoculated on September 22. 1908. when it had been 144 days in artificial cultivation.

1467. SUBCUT.

DOSE, 50 MILLIGRAMMES.



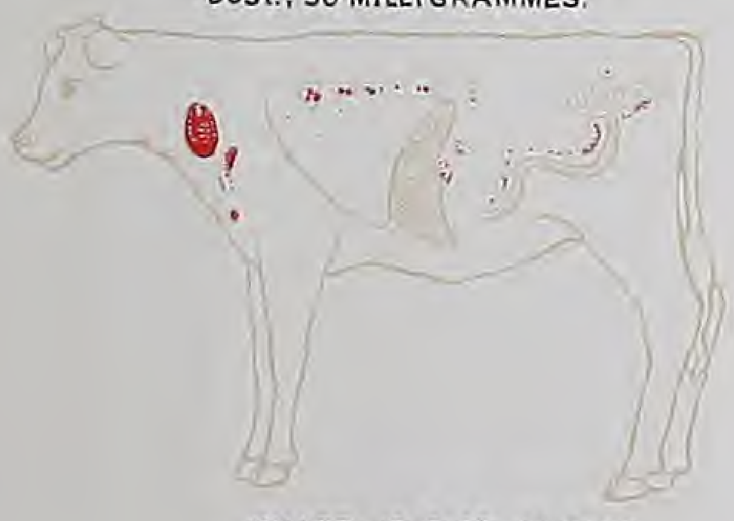
KILLED. 7. 1. 09. 107 DAYS.

VIRUS H. 71. “L.V.”

The strain was derived from the original material through G.P. 1937 and was inoculated on November 9. 1906. when it had been 78 days in artificial cultivation.

1153. SUBCUT.

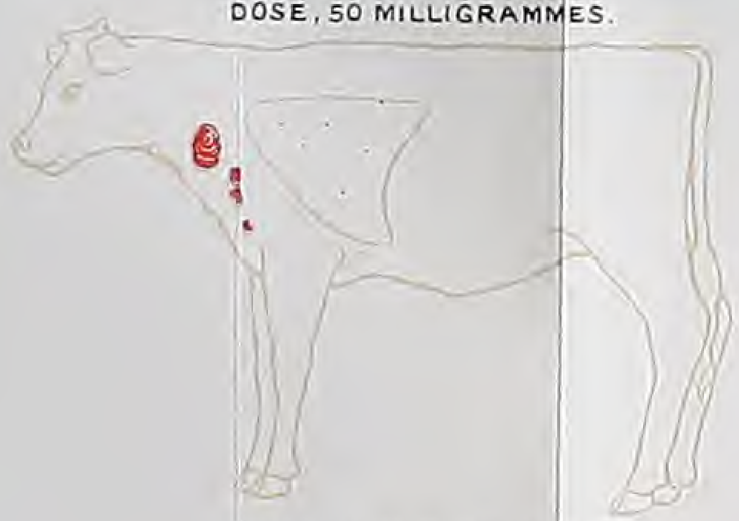
DOSE, 50 MILLIGRAMMES.



KILLED. 12. 2. 07. 95 DAYS.

1163. SUBCUT.

DOSE, 50 MILLIGRAMMES.



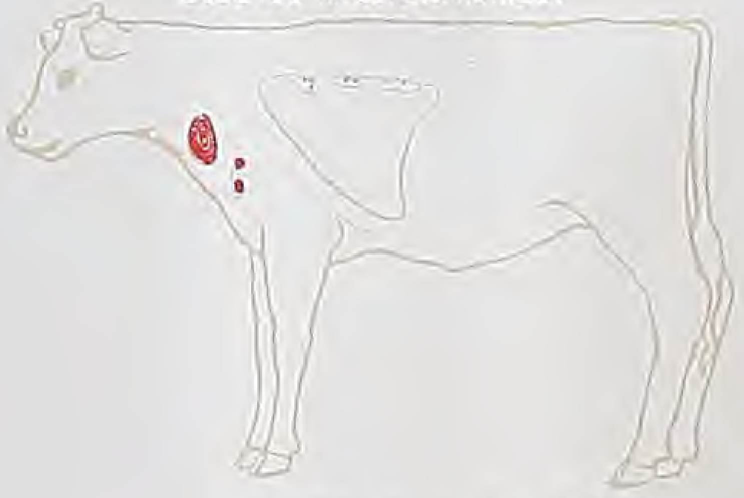
KILLED. 12. 2. 07. 95 DAYS.

VIRUS H. 112. B.B.

The strain was derived from the original material through G.P. 3042 and was inoculated on July 28, 1908. when it had been 63 days in artificial cultivation.

1427. SUBCUT.

DOSE. 50 MILLIGRAMMES.



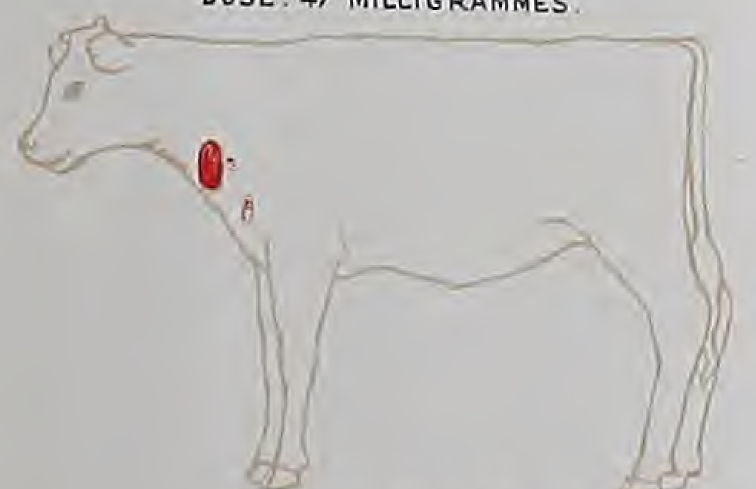
KILLED, 4. 11. 08. 99 DAYS.

VIRUS H 109. “M.W.”

The strain was derived from the original material through G.P. 3002 and was inoculated on June 12, 1908. when it had been 50 days in artificial cultivation.

1437. SUBCUT.

DOSE. 47 MILLIGRAMMES.



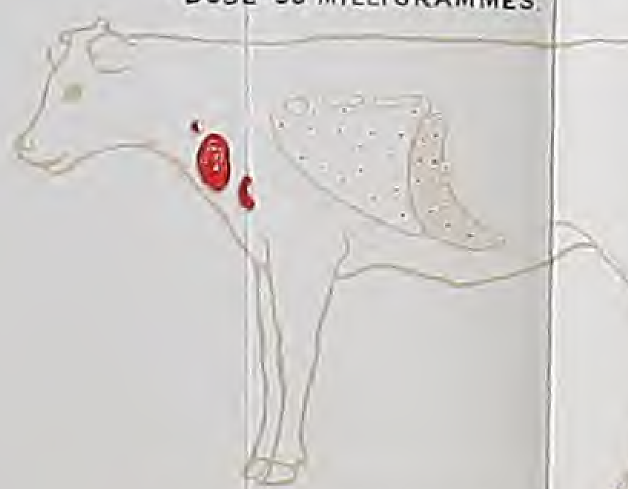
KILLED. 26. 9. 08. 106 DAYS.

VIRUS H. 92. “D.N.”

The strain was derived from the original material through G.P. 2592 and was inoculated on January 2, 1908. when it had been 64 days in artificial cultivation.

1317. SUBCUT.

DOSE 50 MILLIGRAMMES.



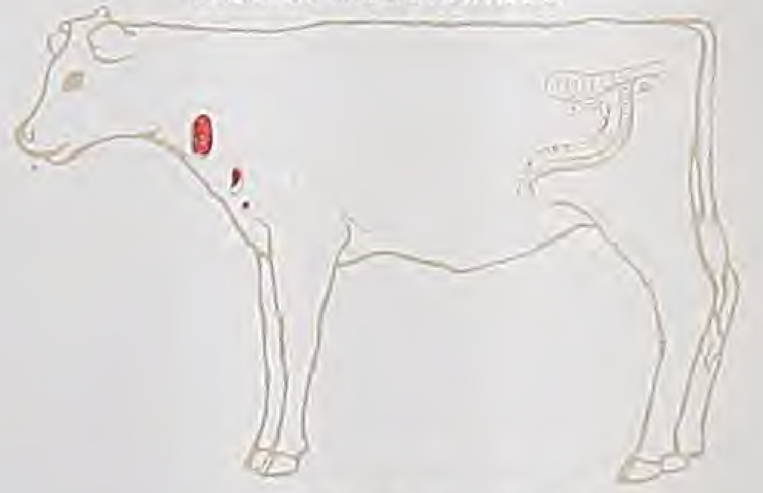
DIED, (RENAL DISEASE) 28. 1. 08. 26 DAYS.

VIRUS H. 99. “L.K.”

The strain was derived from the original material through G.P. 2927 and was inoculated on April 3, 1908. when it had been 28 days in artificial cultivation.

1401. SUBCUT.

DOSE 50 MILLIGRAMMES.



KILLED 1. 8. 08. 120 DAYS.

CHART 37A.

Test Inoculations in Calves and Passage Experiments with the Lupus Virus H. 53. "D.H." (*a*) and (*b*), the Cultures from which grow like Bovine Tubercle Bacillus.

VIRUS H. 53. "D.H." (*a*) and (*b*). *Lupus of the Hip of many years duration.*

Age of patient : { 15 years when the (*a*) strain was obtained.
{ $18\frac{1}{2}$,, ,, (*b*) ,, ,,

The Chart shows diagrammatically the results in Calves (i) of inoculating culture of the (*a*) strain of the virus two years after its isolation, and (ii) of passing this strain through the bodies of calves. The results are seen to be similar to those obtained when the virus was tested nearly two years previously. (*See* Chart 37.)

[For Experiments with H. 53. (*a*) on Pigs and Goats, *see* Chart 39.]

The Chart shows also the experiments made with the (*b*) strain of the virus to determine (i) its original virulence for the calf, and (ii) its virulence after residence in the body of the calf. It will be seen that the virulence of "D.H." (*b*) is apparently higher (for the calf) than that of "D.H." (*a*) but that the virulence was not increased by 'passage.'

References.—Final Report : Pages 16 to 19, 30 and 31.

Appendix to Final Report, Vol. II. : Virus H. 53. "D.H." (*a*), pages 28, 38, 149, 164. Virus H. 53. "D.H." (*b*), pages 28, 43, 156, 219.

PIGS AND GOATS INOCULATED WITH CULTURES FROM THREE LUPUS VIRUSES.

PIGS.

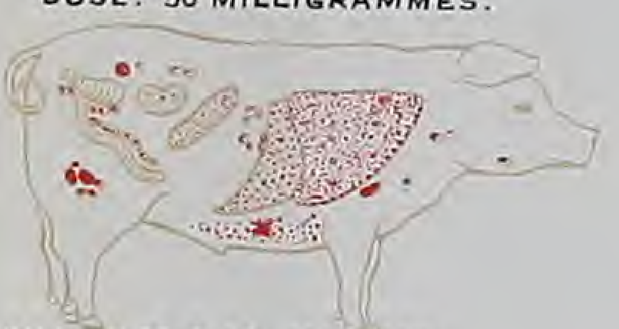
VIRUS H. 53. "D. H" *(a)*

The strain was derived from the original material through G.P. 1482, and was inoculated on February 25, 1907. when it had been 723 days in artificial cultivation.

85. SUBCUT.

(Age 5 Months)

DOSE: 50 MILLIGRAMMES.

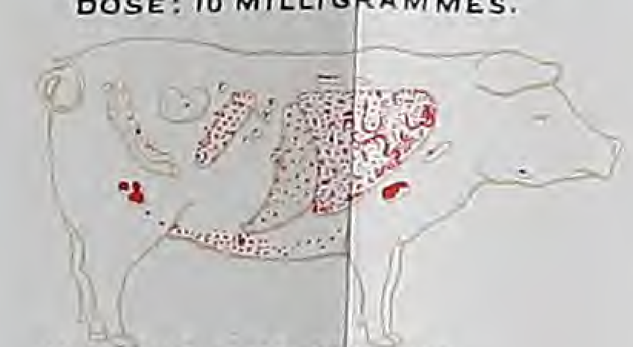


KILLED: 26.2.08. 366 DAYS.

87. SUBCUT.

(Age 5 Months)

DOSE: 10 MILLIGRAMMES.



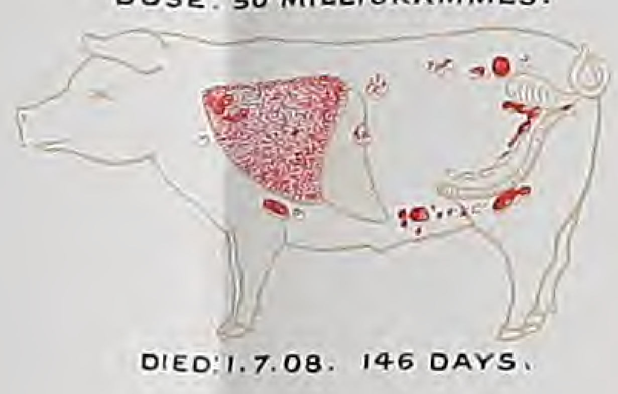
KILLED: 10.10.07. 227 DAYS.

The strain was derived from the original material through G.P. 1482. and was inocalated on February 6. 1908. when it had been 1069 days in artificial cultivation.

121. SUBCUT.

(Age 12½ weeks)

DOSE: 50 MILLIGRAMMES.

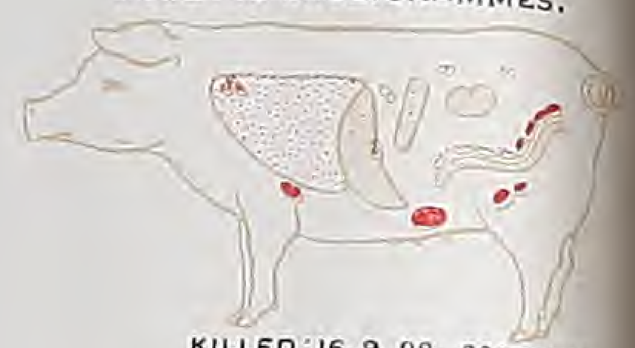


DIED: 1.7.08. 146 DAYS.

119. SUBCUT.

(Age 12½ weeks)

DOSE: 10 MILLIGRAMMES.



KILLED: 16.9.08. 225 DAYS.

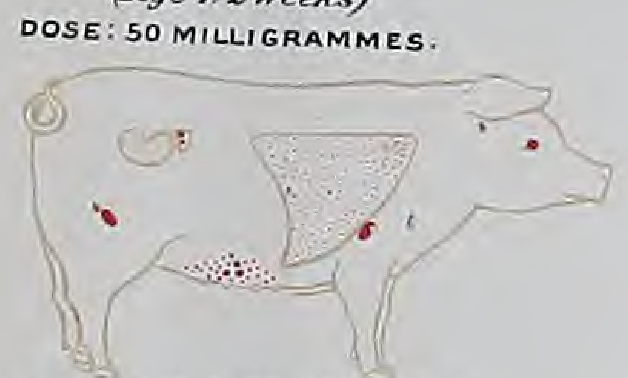
VIRUS H. 92. "D. N".

The strain was derived from the original material through G.P. 2592 and was inoculated on January 2. 1908. when it had been 64 days in artificial cultivation

107. SUBCUT.

(Age 7½ weeks)

DOSE: 50 MILLIGRAMMES.

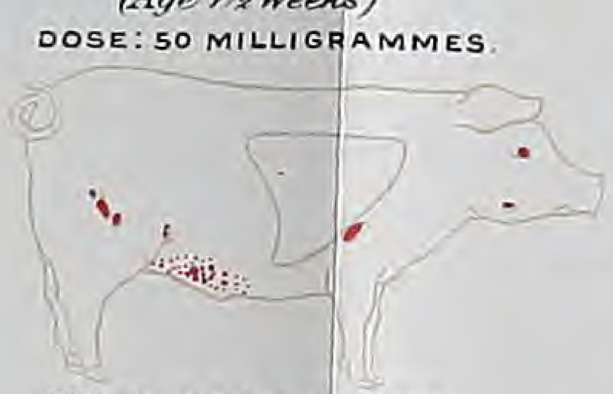


KILLED: 14.5.08. 133 DAYS.

109. SUBCUT.

(Age 7½ weeks)

DOSE: 50 MILLIGRAMMES.



KILLED: 9.6.08. 159 DAYS.

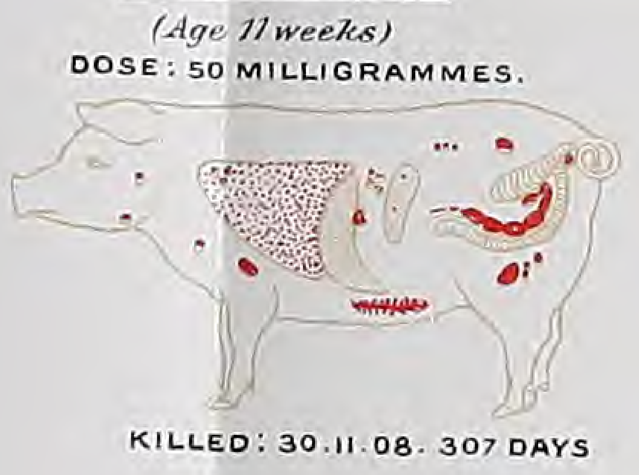
VIRUS H. 85. "H. B".

The strain was derived from the original material through G.P. 2363 and was inoculated on January 28. 1908 when it had been 244 days in artificial cultivation.

115. SUBCUT.

(Age 11 weeks)

DOSE: 50 MILLIGRAMMES.

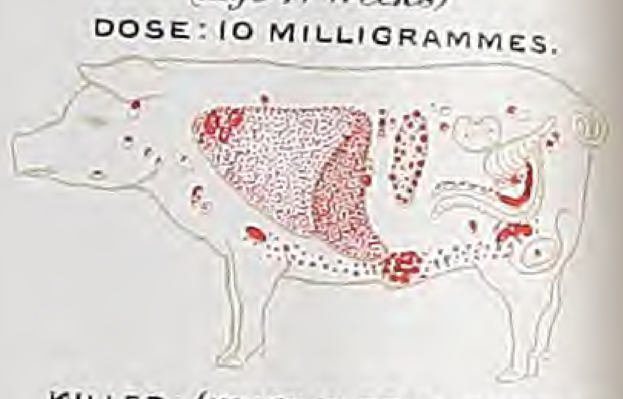


KILLED: 30.11.08. 307 DAYS

117. SUBCUT.

(Age 11 weeks)

DOSE: 10 MILLIGRAMMES.



KILLED (WHEN IN GOOD CONDITION) 22.10.08. 268 DAYS.

GOATS.

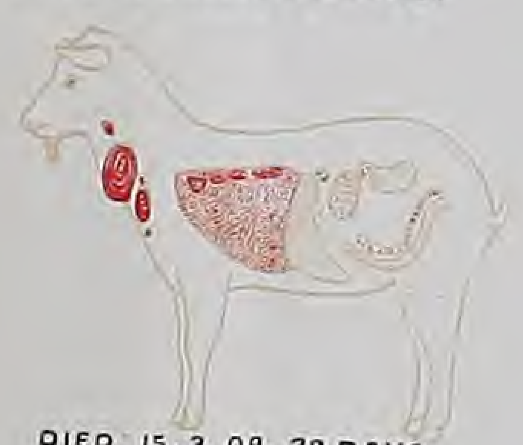
VIRUS H. 53. "D. H" *(a)*

The strain was derived from the original material through G.P. 1482, and was inoculated on February 6. 1908. when it had been 1069 days in artificial cultivation.

65. SUBCUT.

(Kid)

DOSE: 10 MILLIGRAMMES.



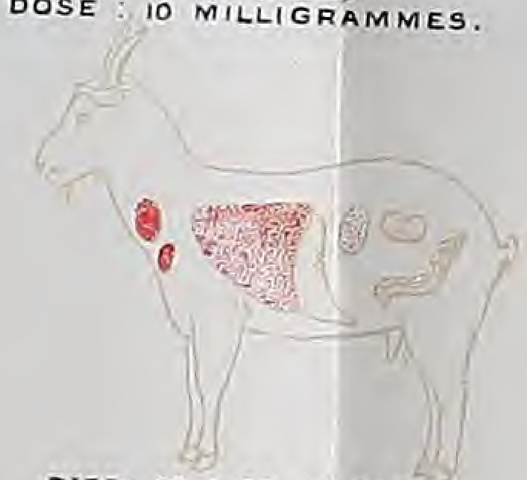
DIED: 15.3.08. 38 DAYS.

The strain was derived from the original material through G.P. 1482. and was inoculated on April 1, 1908. when it had been 1124 days in artificial cultivation.

67. SUBCUT.

(Adult)

DOSE: 10 MILLIGRAMMES.



DIED: 30.5.08. 59 DAYS

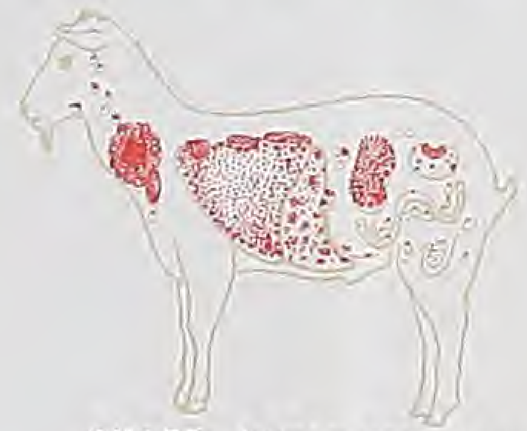
VIRUS H. 85. "H. B".

The strain was derived from the original material through G.P. 2363. and was inoculated on January 28. 1908. when it had been 244 days in artificial cultivation

63. SUBCUT.

(Kid)

DOSE: 10 MILLIGRAMMES.



KILLED (WHEN VERY ILL) 5.3.09. 402 DAYS.

PIGS AND GOATS INOCULATED WITH GROUP II CULTURES.

(INCLUDED FOR COMPARISON WITH THE ABOVE)

PIGS.

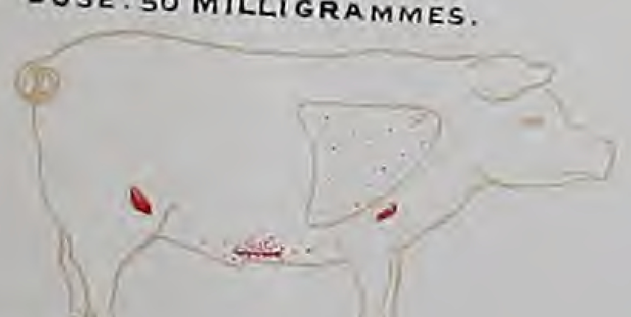
VIRUS H. 93. "C. D".

The strain was derived from the human lung through G.P. 2610. and was inoculated on January 2, 1908. when it had been 78 days in artificial cultivation.

111. SUBCUT.

(Age 7½ weeks)

DOSE: 50 MILLIGRAMMES.

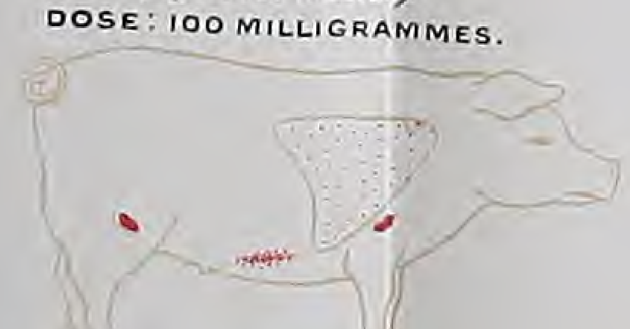


KILLED: 10.9.08. 252 DAYS.

113. SUBCUT.

(Age 7½ weeks)

DOSE: 100 MILLIGRAMMES.



KILLED: 11.9.08. 253 DAYS.

GOATS.

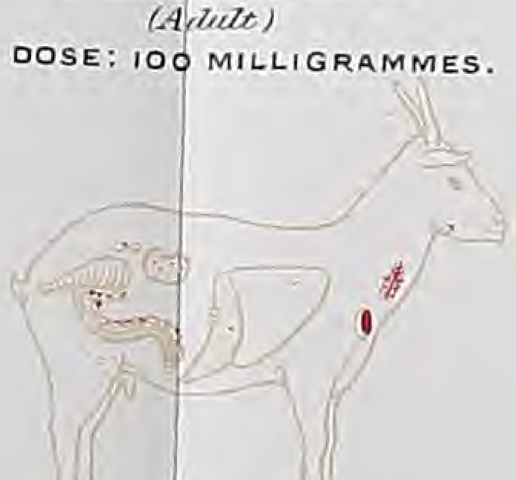
VIRUS H. 79. "J. N".

The strain was derived from the human lung, direct, and was inoculated on November 28, 1907. when it had been 335 days in artificial cultivation.

59. SUBCUT.

(Adult)

DOSE: 100 MILLIGRAMMES.



KILLED: 13.3.08. 106 DAYS.

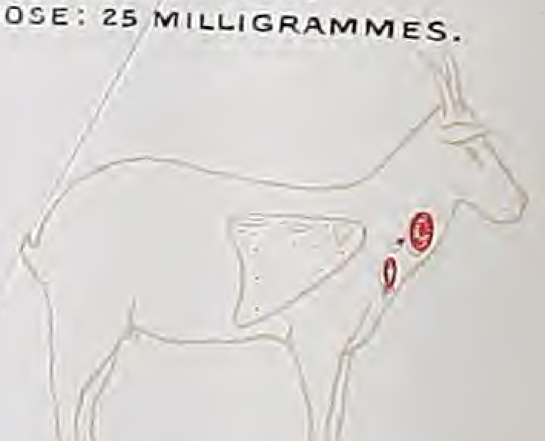
VIRUS H. 118. "F. C".

The strain was derived from the original material (human sputum) through G.P. 3313. and was inoculated on February 12, 1909. whe it had been 84 days in artificial cultivation.

77. SUBCUT.

(Young adult)

DOSE: 25 MILLIGRAMMES.



KILLED: 8.6.09. 116 DAYS.

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CHART 38.

"Culture Inoculations in Calves of the Eleven Easy-growing Lupus Viruses."

[*See* Appendix to Final Report, Vol. II., page 29.]

VIRUS H. 102. "N.H." *Lupus of the face and arm; duration of disease 15 years.* The culture was derived from the arm.

Age of patient: 33 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 55, 159, and 345.

VIRUS H. 103. "N.S." *Lupus of the leg (following tuberculous ulceration of foot); duration of disease 24 years.* Culture derived from the leg.

Age of patient: 28 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 56 and 355.

VIRUS H. 106. "K.R." *Lupus of the face, ear, neck, and arm; duration of disease about 20 years.* The culture was derived from the elbow.

Age of patient: 27 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 59 and 391.

VIRUS H. 84. "M.S." *Lupus of the cheek; duration of disease 15 years.*

Age of patient: 68 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 45, 160, and 255.

VIRUS H. 101. "E.G." *Lupus of the cheeks, nose, arm, and wrist; duration of disease 36 years.* The culture was derived from the elbow.

Age of patient: 53 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 54 and 339.

VIRUS H. 114. "A.M." *Lupus of the nose and neck; duration of disease 15 years.* The culture was derived from the neck.

Age of patient: 16 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: pages 68, 160, and 473.

VIRUS H. 71. "L.V." (*a*). *Lupus of the face, neck, back, arm, and hand; duration of disease 9 years.* The culture was derived from the arm.

Age of patient: 14 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 14, 159, and 245.

NOTE.—Two years later material was again obtained by operation from the same patient. A culture raised from it was found to be identical in cultural characters and virulence with the (*a*) strain. (*See* H. 71. "L.V." (*b*) in Vol. II., of Appendix, page 14.)

VIRUS H. 112. "B.B." *Lupus of the arm: duration of disease 7–8 years.*

Age of patient: 16 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 67 and 469.

VIRUS H. 109. "M.W." *Lupus of the elbows; duration of disease 3 years.* The culture was derived from the right elbow.

Age of patient: 5 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 63 and 443.

VIRUS H. 92. "D.N." *Lupus of the cheek; duration of disease $1\frac{2}{5}$ years.*

Age of patient: $3\frac{3}{4}$ years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 50 and 303.

VIRUS H. 99. "L.K." *Lupus of the upper lip, nose, and neck; duration of disease not known.* The culture was derived from the nose.

Age of patient: 8 years.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pages 52 and 313.

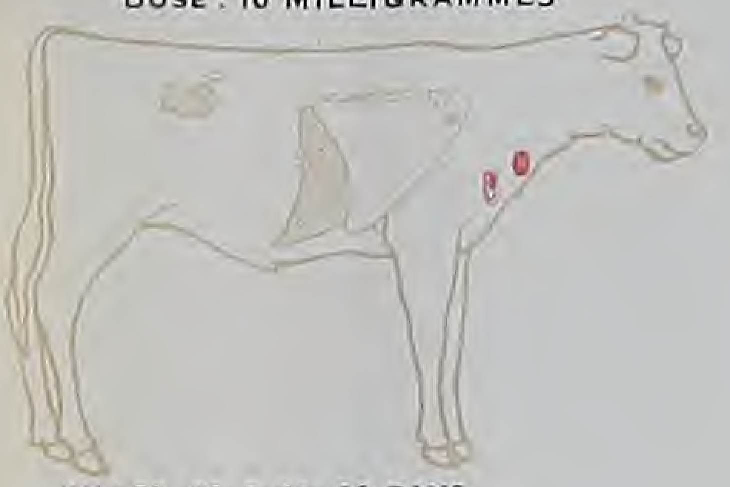
INOCULATION EXPERIMENTS ON CALVES WITH CULTURE OF VIRUS E.II.

PASSAGE EXPERIMENTS α AND β

The strain was derived from the original material through a guineapig, and was inoculated on April 30.1906. when it had been 130 days in artificial cultivation.

404. SUBCUT.

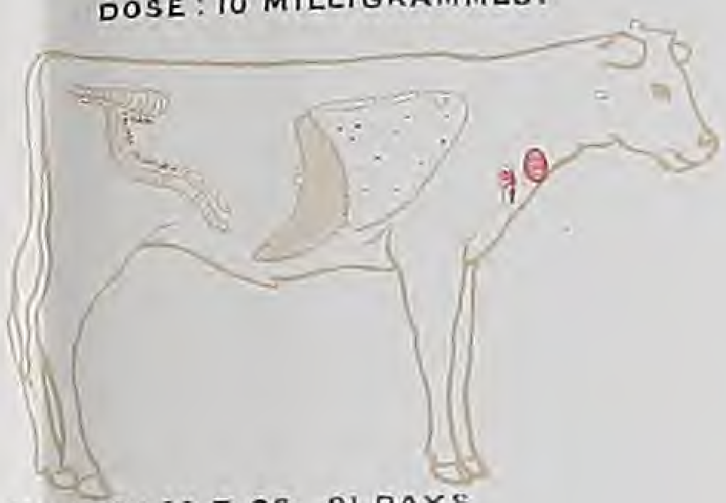
DOSE: 10 MILLIGRAMMES



KILLED 28.7.06. 89 DAYS

406. SUBCUT.

DOSE: 10 MILLIGRAMMES.



KILLED: 30.7.06. 91 DAYS

CULTURE

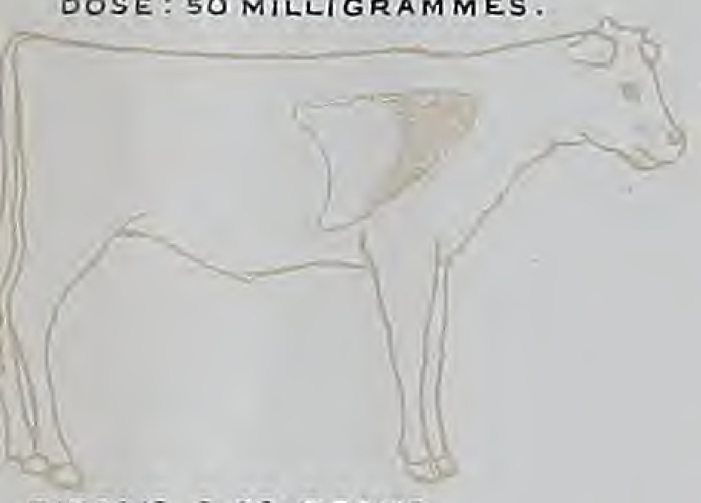
Derived from the prescapular gland of Calf 404; inoculated on February 12. 1908. after 564 days artificial cultivation.

CULTURE

Derived from the prescapular gland of Calf 406; inoculated on February 18. 1908. after 568 days artificial cultivation.

590. SUBCUT.

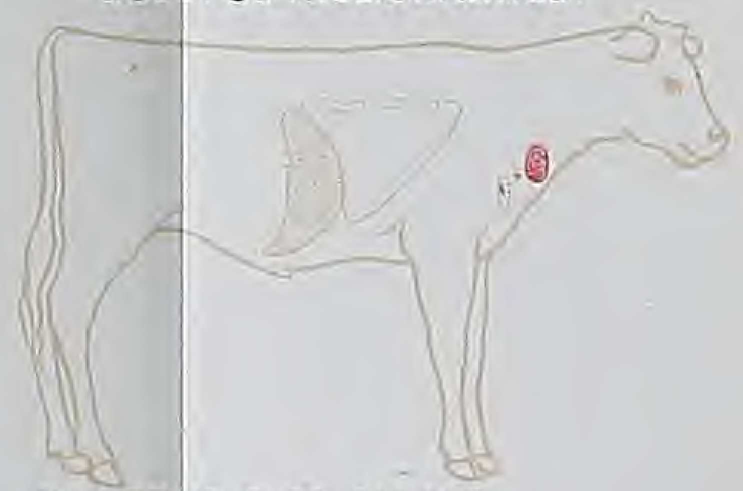
DOSE: 50 MILLIGRAMMES.



DIED: 19.2.08. 7 DAYS.
(BRONCHO-PNEUMONIA)

598. SUBCUT.

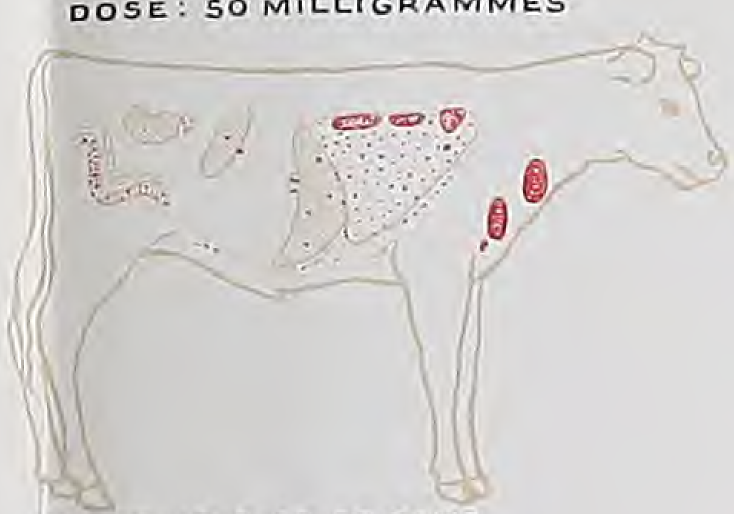
DOSE: 50 MILLIGRAMMES.



KILLED: 13.5.08. 91 DAYS.

596. SUBCUT.

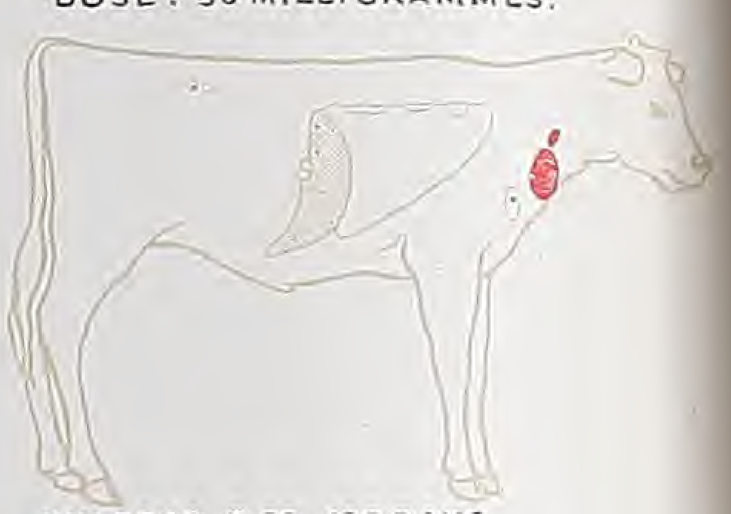
DOSE: 50 MILLIGRAMMES



KILLED: 18.5.08. 90 DAYS.

600. SUBCUT.

DOSE: 50 MILLIGRAMMES.



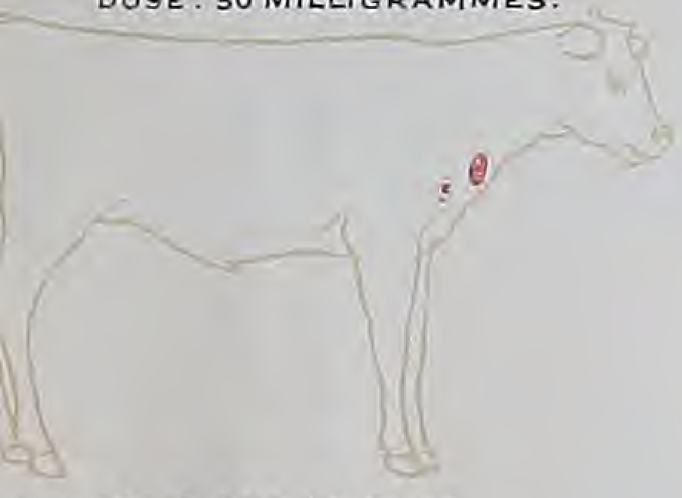
KILLED: 3.7.08. 136 DAYS.

CULTURE

erived from the spleen of Calf 590; noculated on April 29.1908. after 0 days artificial cultivation.

618. SUBCUT.

DOSE: 50 MILLIGRAMMES.



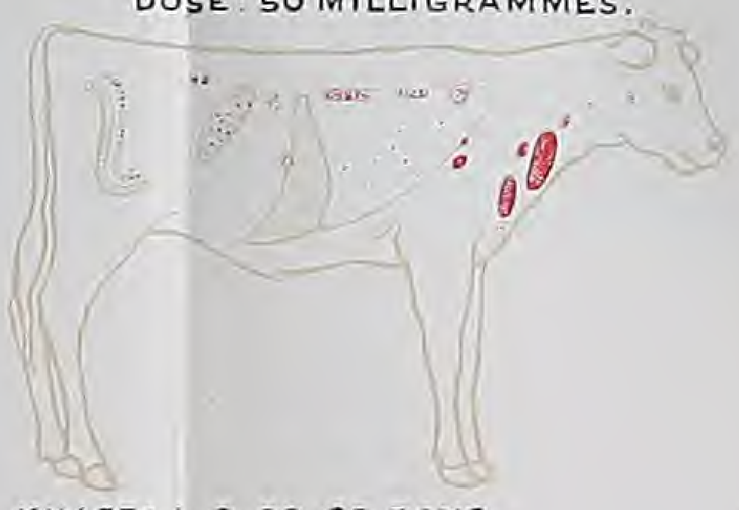
KILLED: 22.7.08. 84 DAYS.

CULTURE

Derived from the prescapular gland of Calf 598; inoculated on May 29.1908. after 16 days artificial cultivation.

640. SUBCUT

DOSE: 50 MILLIGRAMMES.



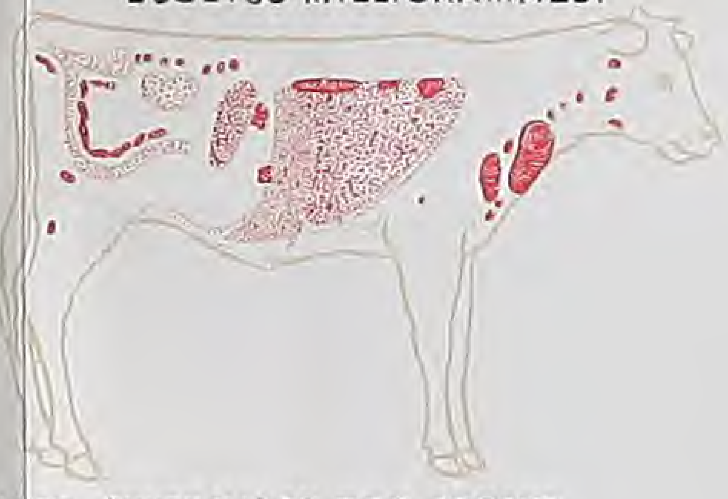
KILLED: 1.9.08. 95 DAYS

CULTURE

Derived from the lung of Calf 596; inoculated on June 10, 1908, after 23 days artificial cultivation.

638. SUBCUT.

DOSE: 50 MILLIGRAMMES.



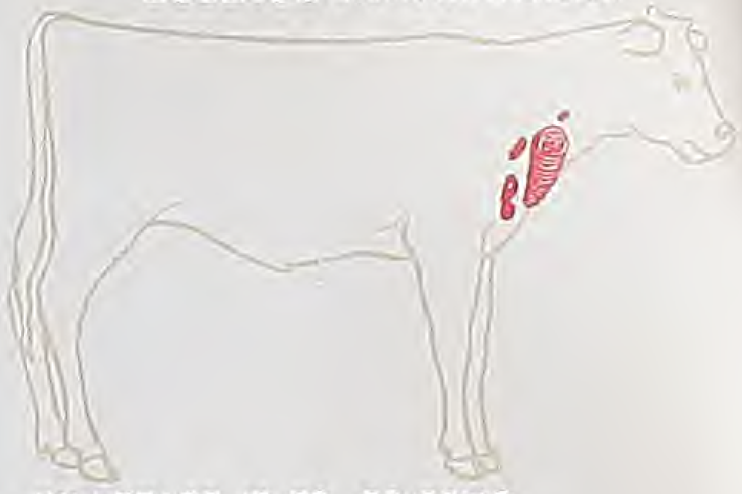
KILLED (WHEN ILL) 20.7.08. 40 DAYS.

CULTURE

Derived from the prescapular gland of Calf 600; inoculated on August 4, 1908. after 32 days artificial cultivation.

648. SUBCUT.

DOSE: 50 MILLIGRAMMES.



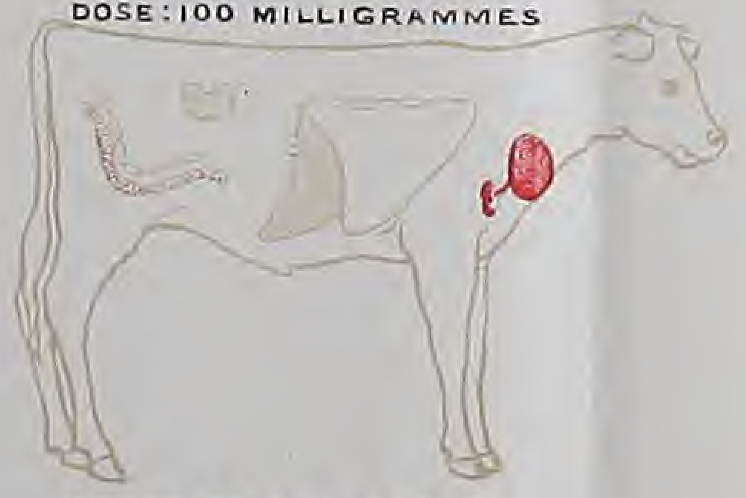
KILLED: 26.10.08. 83 DAYS.

INOCULATION EXPERIMENTS.

The strain was derived from the original material through a guineapig, and was inoculated on April, 10, 1908. when it had been 841 days in artificial cultivation

608. SUBCUT.

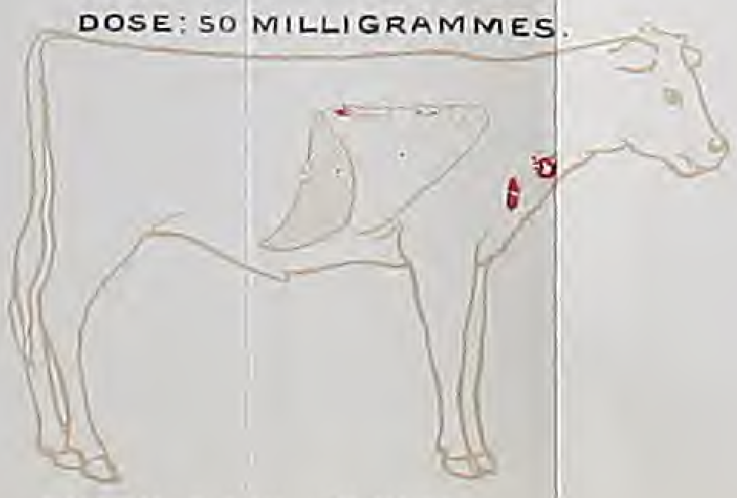
DOSE: 100 MILLIGRAMMES



KILLED: 29.6.08. 80 DAYS

606. SUBCUT.

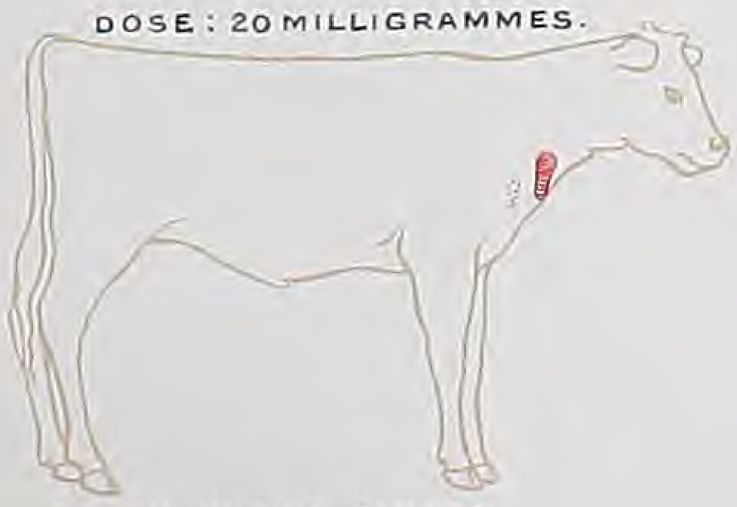
DOSE: 50 MILLIGRAMMES.



KILLED: 8.7.08. 89 DAYS

610. SUBCUT.

DOSE: 20 MILLIGRAMMES.



KILLED: 10.7.08. 91 DAYS.

CHART 39.

Pigs and Goats Inoculated wih Cultures from Three Lupus Viruses.

Inoculation experiments on Calves with these viruses are charted elsewhere in the series: Virus H. 53. "D.H." (*a*). *See* Chart 37; Virus H. 85. "H.B." *See* Chart 36; Virus H. 92. "D.N." *See* Chart 38.

References.—Final Report: Pages 16 to 19.

Appendix to Final Report, Vol. II.: Pigs: Pages 18, 119–120. Goats: Pages 19, 121–122; *see* also Summaries on pages 39 and 40, 47 and 51.

Pigs and Goats Inoculated with Group II. Cultures (included for Comparison with the above).

Inoculation experiments on calves with these slightly virulent viruses have not been charted in this series. They were shown to be identical in virulence with the viruses on Chart 33.

VIRUS H. 93. "C.D." *General miliary Tuberculosis.*

Age of patient: 2¼ years.

Material used: Culture from *Lung.*

VIRUS H. 79. "J.N." *Pulmonary Tuberculosis.*

Age of patient: 24 years.

Material used: Culture from *Lung.*

VIRUS H. 118. "F.C." *Pulmonary Tuberculosis.*

Age of patient: 50 years.

Material used: Culture from *Sputum.*

References.—Final Report: Pages 9 to 13.

Appendix to Final Report, Vol. I.: Pigs: Pages 44 and 240. Goats: Pages 44 and 241; *see* also Summaries on pages 123, 136, and 144.

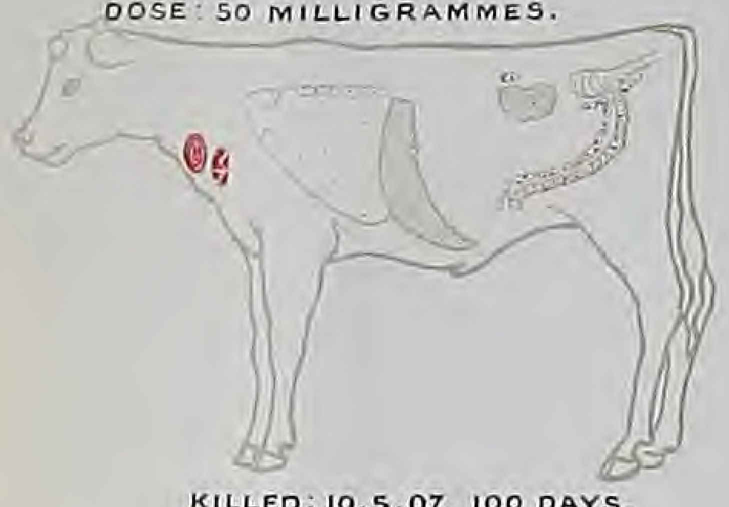
INOCULATION EXPERIMENTS ON CALVES WITH CULTURE OF VIRUS E. II.

PASSAGE EXPERIMENTS γ AND δ.

The strain was derived from the original material through a guineapig, and was inoculated on January 30, 1907. when it had been 405 days in artificial cultivation.

504. SUBCUT.

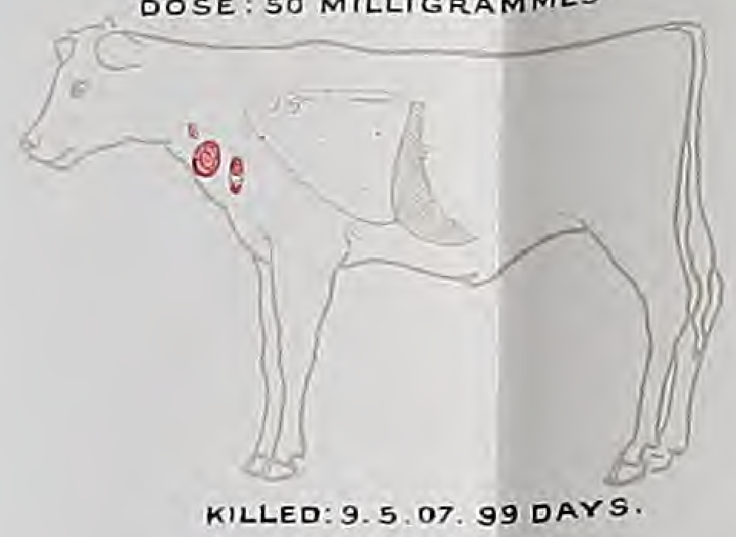
DOSE: 50 MILLIGRAMMES.



KILLED: 10.5.07. 100 DAYS.

506. SUBCUT.

DOSE: 50 MILLIGRAMMES



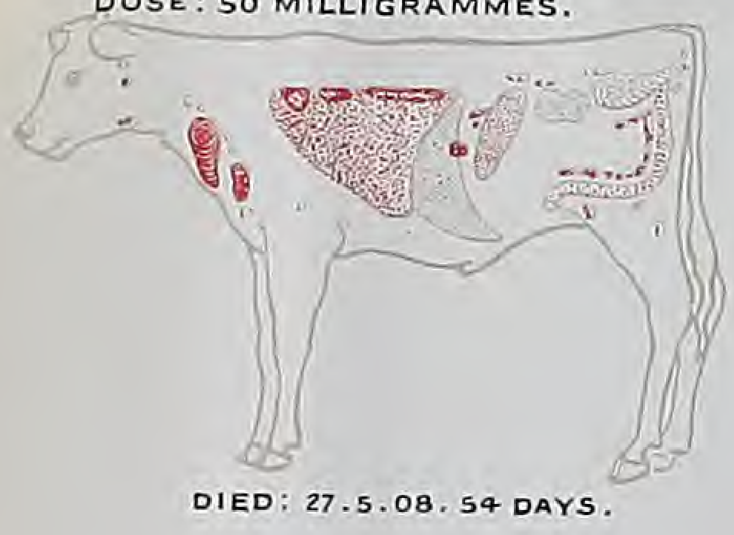
KILLED: 9.5.07. 99 DAYS.

CULTURE

Derived from the prescapular gland of Calf 504; inoculated on April 3. 1908. after 329 days artificial cultivation.

626. SUBCUT.

DOSE: 50 MILLIGRAMMES.



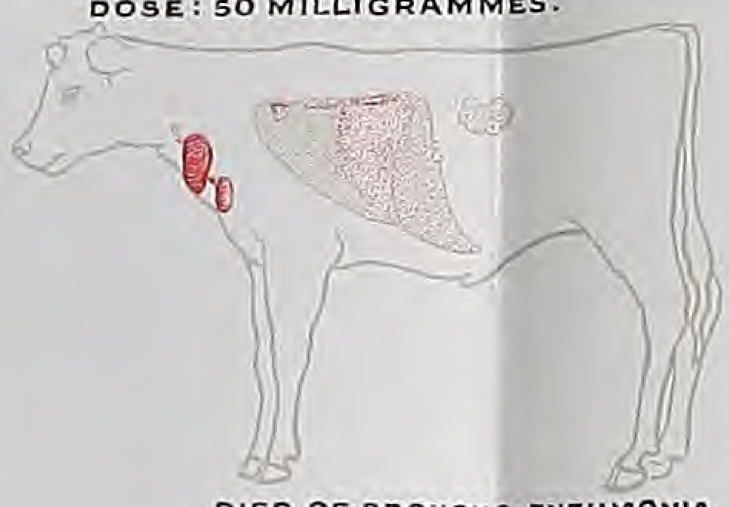
DIED: 27.5.08. 54 DAYS.

CULTURE

Derived from the lung of Calf 506; inoculated on February 18. 1908. after 285 days artificial cultivation.

594. SUBCUT.

DOSE: 50 MILLIGRAMMES.



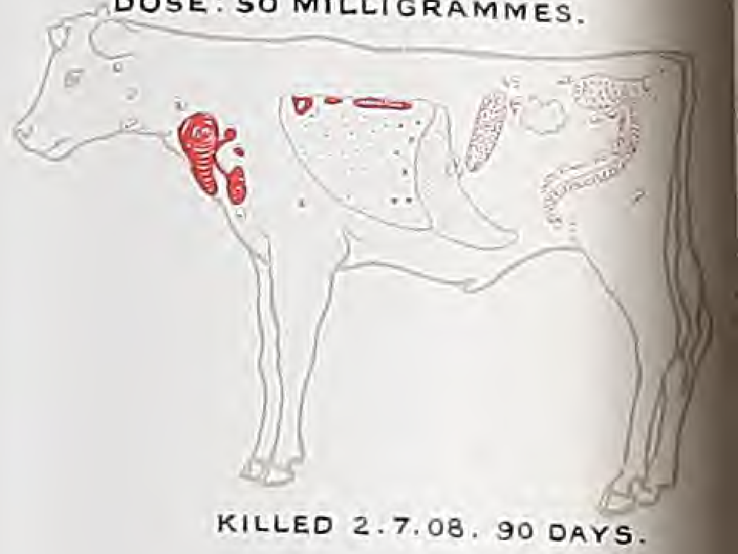
DIED OF BRONCHO-PNEUMONIA.
5.3.08. 16 DAYS.

CULTURE

Derived from the lung of Calf 506; inoculated on April 3. 1908. after 330 days artificial cultivation.

628. SUBCUT.

DOSE: 50 MILLIGRAMMES.



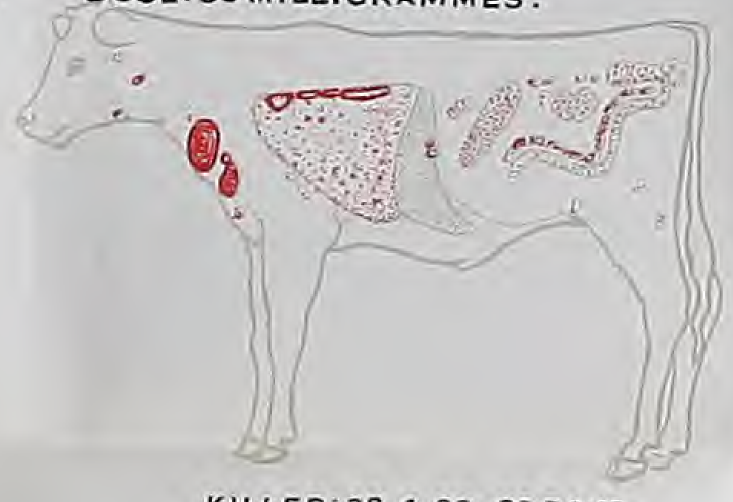
KILLED 2.7.08. 90 DAYS.

CULTURE

Derived from the lung of Calf 504; inoculated on January 29, 1908. after 264 days artificial cultivation.

578. SUBCUT.

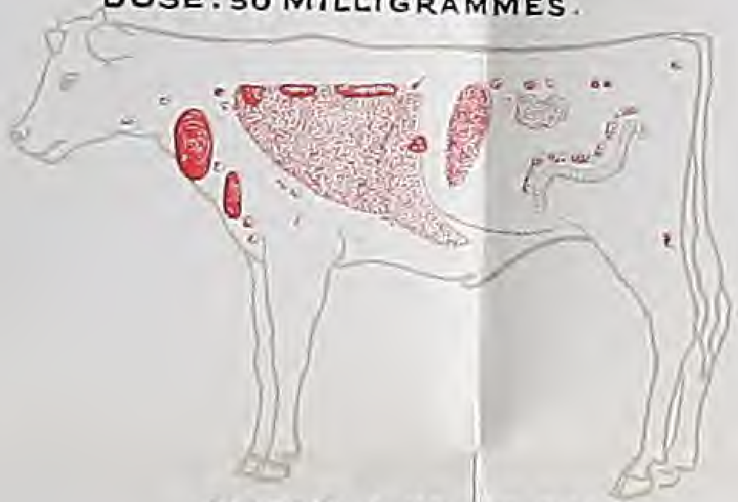
DOSE: 50 MILLIGRAMMES.



KILLED: 28.4.08. 90 DAYS.

580. SUBCUT.

DOSE: 50 MILLIGRAMMES.



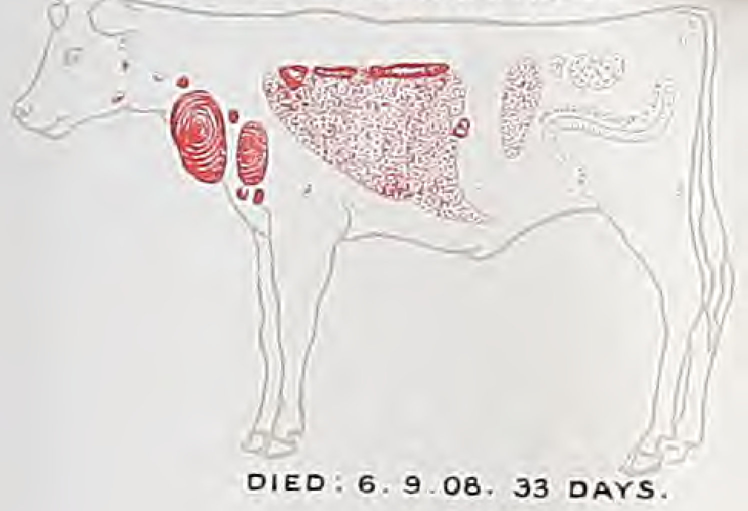
DIED: 27.2.08. 29 DAYS.

CULTURE

Derived from the lung of Calf 628; inoculated on August 4, 1908. after 33 days artificial cultivatio

646. SUBCUT.

DOSE: 50 MILLIGRAMMES.



DIED: 6.9.08. 33 DAYS.

RABBIT PASSAGE EXPERIMENT.

1330. SUBCUT AND INTRAP.

Inoculated on May 15, 1907. with 1 Milligramme of culture derived from the original material. Died of chronic general tuberculosis in 265 days.

CULTURE

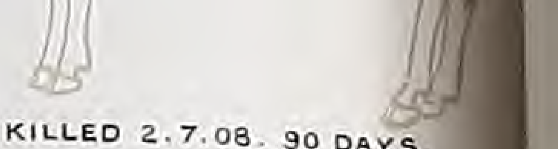
Derived from the Diaphragm of Rabbit 1330; inoculated on May 11, 1908, after 97 days artificial cultivation

632. SUBCUT.

DOSE: 50 MILLIGRAMMES.

DIED: 27.5.08. 54 DAYS.

DIED OF BRONCHO-PNEUMONIA.
5.3.08. 16 DAYS.



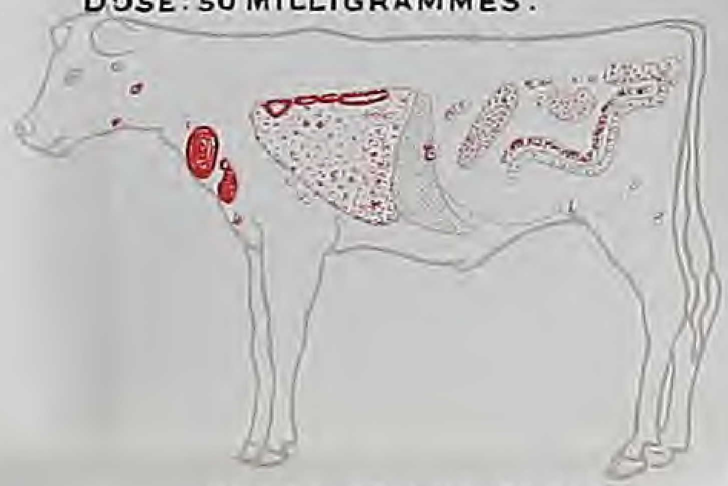
KILLED 2.7.08. 90 DAYS.

CULTURE

Derived from the lung of Calf 504;
inoculated on January 29, 1908.
after 264 days artificial cultivation.

578. SUBCUT.

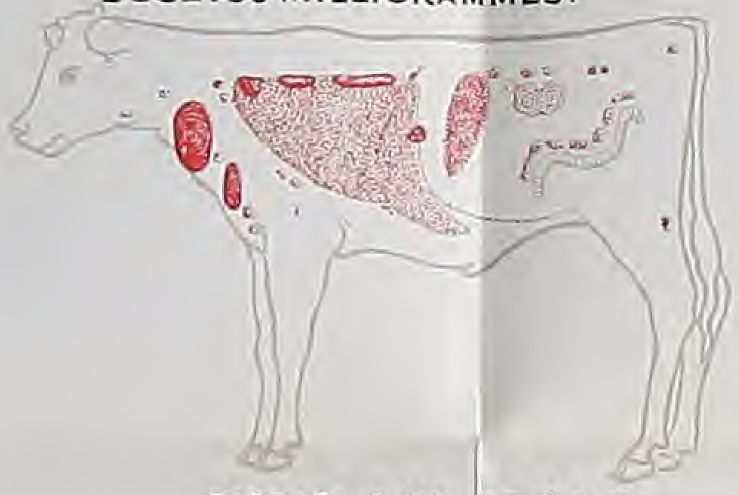
DOSE: 50 MILLIGRAMMES.



KILLED: 28.4.08. 90 DAYS.

580. SUBCUT.

DOSE: 50 MILLIGRAMMES.



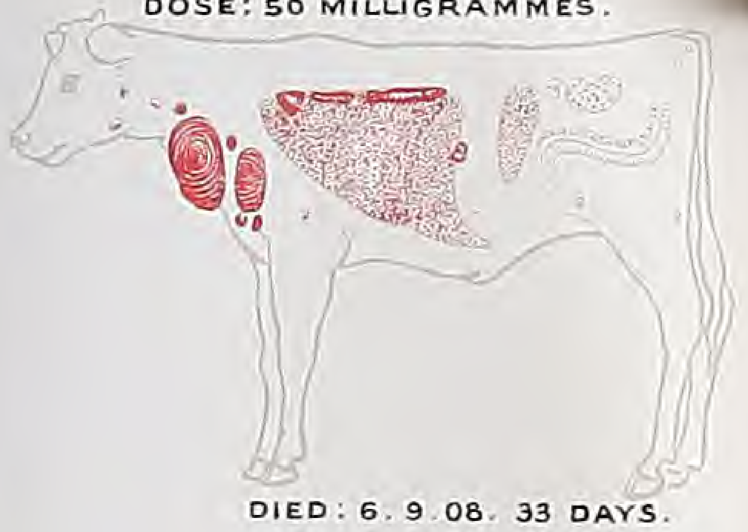
DIED: 27.2.08. 29 DAYS.

CULTURE

Derived from the lung of Calf
628; inoculated on August 4, 1908.
after 33 days artificial cultivation

646. SUBCUT.

DOSE: 50 MILLIGRAMMES.



DIED: 6.9.08. 33 DAYS.

RABBIT PASSAGE EXPERIMENT.

1330. SUBCUT AND INTRAP.

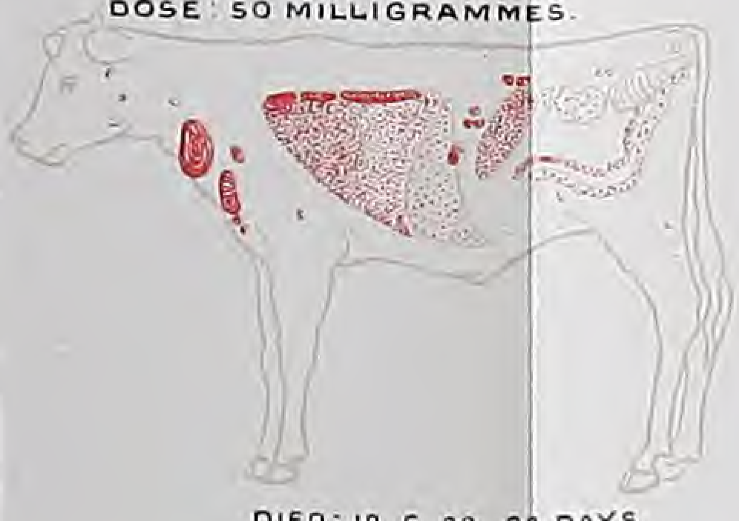
Inoculated on May 15, 1907. with
1 Milligramme of culture derived
from the original material. Died of
chronic general tuberculosis in
265 days.

CULTURE

Derived from the Diaphragm of
Rabbit 1330; inoculated on May 11,
1908. after 97 days artificial
cultivation

632. SUBCUT.

DOSE: 50 MILLIGRAMMES.



DIED: 18.6.08. 38 DAYS.

CHARTS 40 and 40A.

Inoculation Experiments on Calves with Culture of Virus E. II.

[*See* Appendix to Final Report, Vol. IV.: "Investigation of Tubercle Bacilli derived from Tuberculosis occurring naturally in the Horse.

Virus E. II. was obtained on December 19, 1905, from the mesenteric gland of a horse, which showed on post-mortem examination tuberculous lesions in the mesenteric glands and spleen.

Explanation of Charts.—Chart 40 shows diagrammatically the effect on (five) calves (Nos. 404 and 406, and 606, 608, and 610) of inoculation of the original culture. Also it shows the results obtained in two, (α) and (β), of the four passage experiments undertaken to determine whether the original low virulence of the virus for the calf was stable or capable of being raised by residence in the bodies of successive calves.

Chart 41 shows the effect on other (two) calves (Nos. 504 and 506) of inoculation of the original culture, as also the results obtained with the additional passage experiments (γ) and (δ).

Chart 40A also shows that the virulence of the virus for the calf (Calf 632) was increased by residence in the body of the rabbit. (*See* "Rabbit Passage Experiment.")

References.—Final Report: Pages 21 to 24, and 31.

Appendix to Final Report, Vol. IV.: Pages 7, 10, 12, 47 to 66, and 105 to 122.

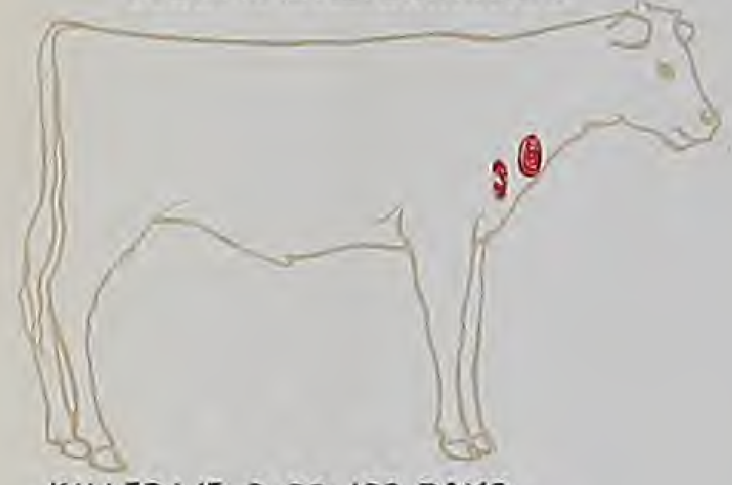
INOCULATION EXPERIMENTS ON CALVES AND BABOONS WITH CULTURE OF VIRUS E. IV.

PASSAGE EXPERIMENT α

The strain was derived from the original material through a guineapig, and was inoculated on April 30. 1906 when it had been 115 days in artificial cultivation.

408. SUBCUT.

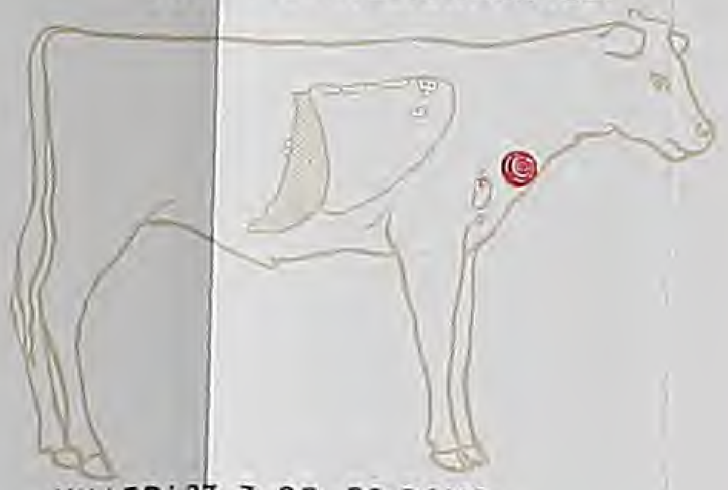
DOSE: 10 MILLIGRAMMES



KILLED: 17. 8. 06. 109 DAYS

410. SUBCUT.

DOSE: 10 MILLIGRAMMES.



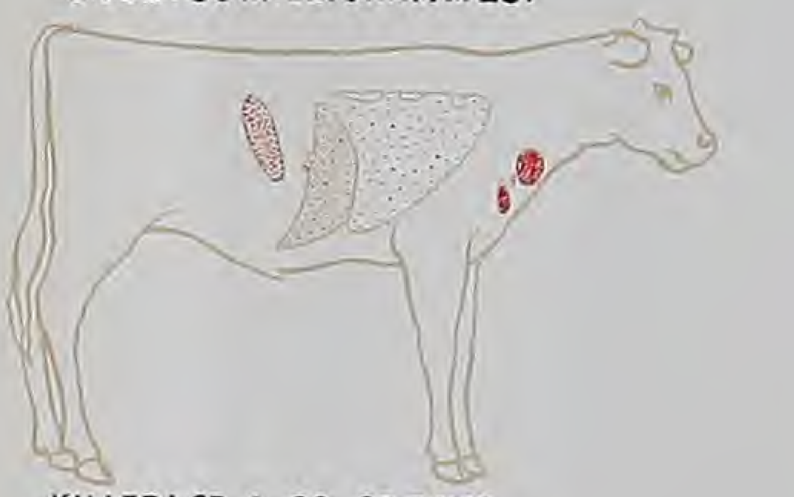
KILLED: 27. 7. 06. 88 DAYS.

CULTURE

Derived from the prescapular gland of Calf 408; inoculated on January 30. 1908, after 531 days artificial cultivation.

582. SUBCUT.

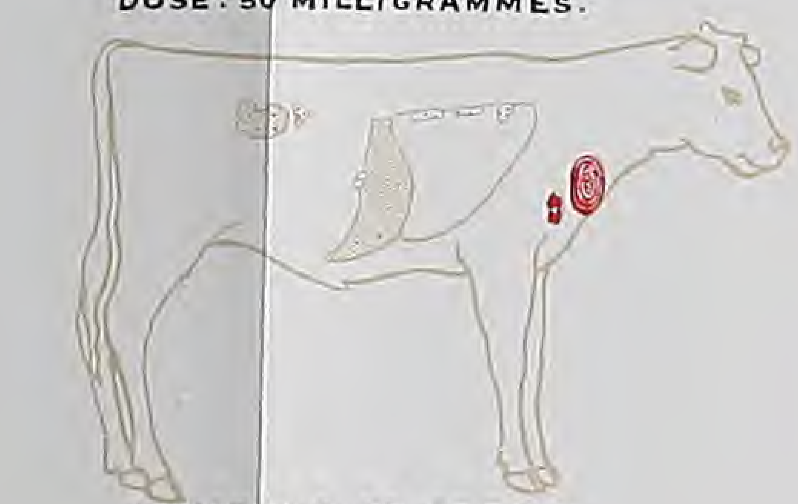
DOSE: 50 MILLIGRAMMES.



KILLED: 27. 4. 08. 88 DAYS

584. SUBCUT.

DOSE: 50 MILLIGRAMMES.



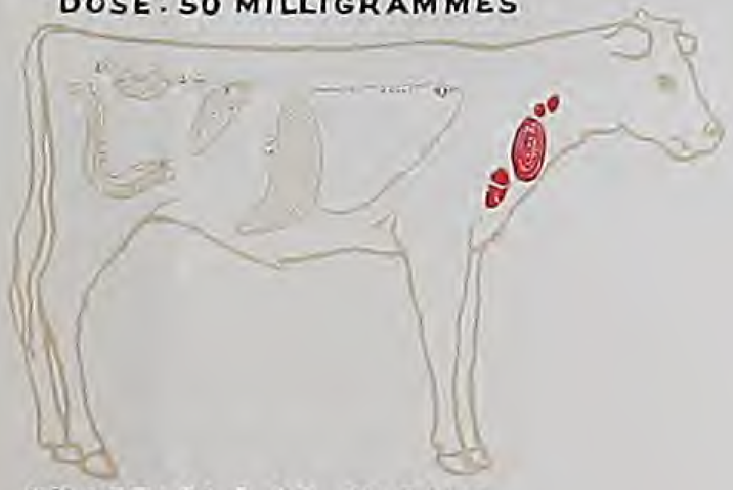
KILLED: 7. 5. 08. 98 DAYS.

CULTURE

Derived from the spleen of Calf 582; inoculated on May 25. 1908, after 28 days artificial cultivation.

636. SUBCUT

DOSE: 50 MILLIGRAMMES



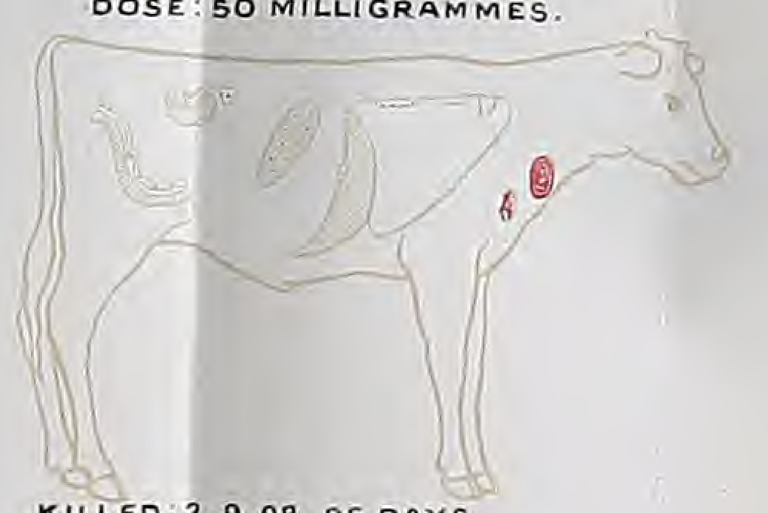
KILLED: 31. 8. 08. 98 DAYS

CULTURE

Derived from the suprarenal body of Calf 584; inoculated on May 29, 1908. after 22 days artificial cultivation.

642. SUBCUT.

DOSE: 50 MILLIGRAMMES.



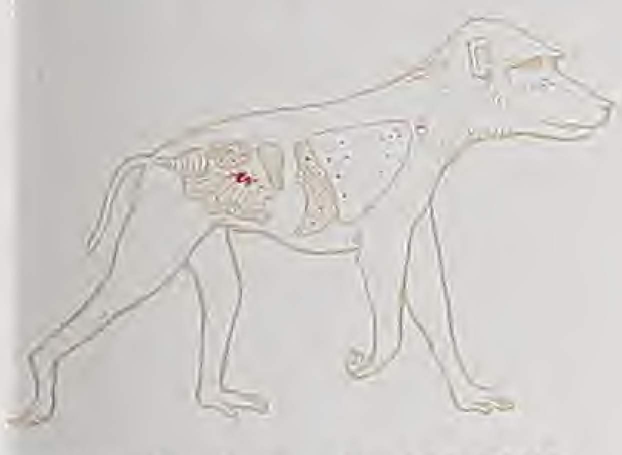
KILLED: 2. 9. 08. 96 DAYS.

EXPERIMENTS ON BABOONS AND CALVES.

The strain was derived from the original material through a guineapig, and was inoculated on November 21. 1906. when it had been 320 days in artificial cultivation.

74. FED.

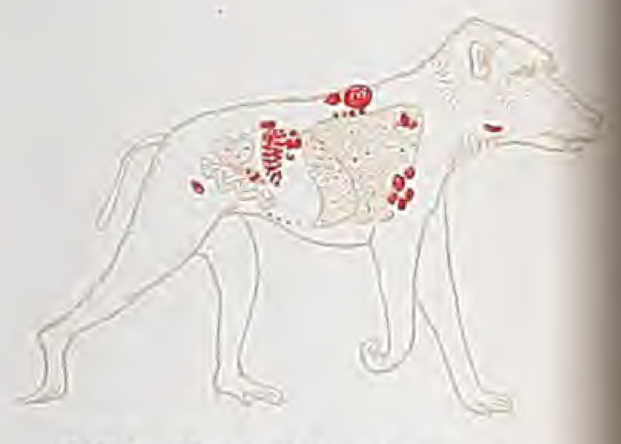
DOSE: 1·0 MG



KILLED: 12. 2. 08. 446 DAYS

72. SUBCUT.

DOSE: 1·0 MG.



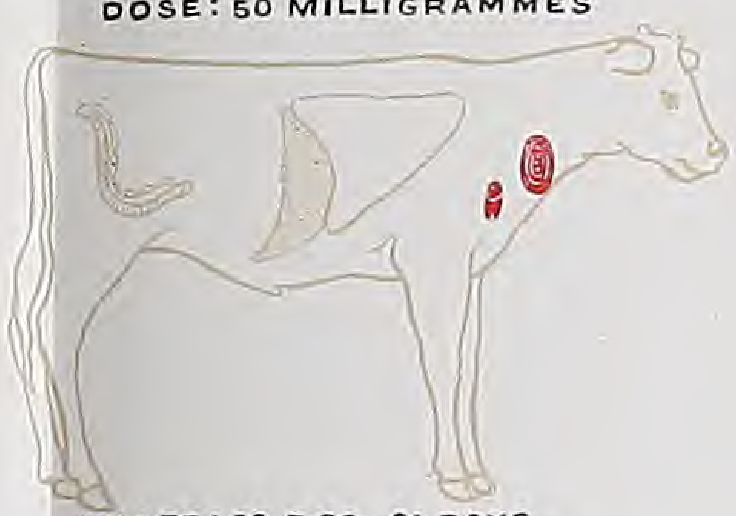
DIED 14. 4. 07. 144 DAYS.

CULTURE

Derived from a mesenteric gland of baboon 74; inoculated on April 28, 1908. after 76 days artificial cultivation.

604. SUBCUT.

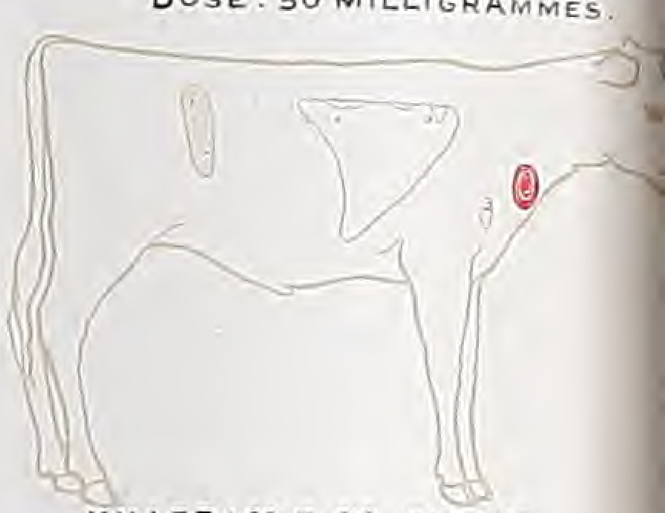
DOSE: 50 MILLIGRAMMES



KILLED: 28. 7. 08. 91 DAYS

612. SUBCUT.

DOSE: 50 MILLIGRAMMES.



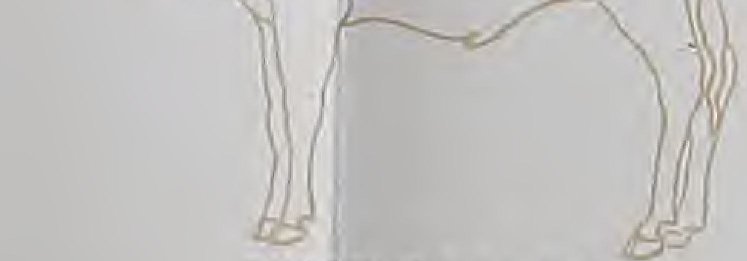
KILLED: 28. 7. 08. 91 DAYS.

INOCULATION EXPERIMENTS.

The strain was derived from the original material through a guineapig, and was inoculated on March 11, 1908. when it had been 795 days in artificial cultivation.

616. SUBCUT.

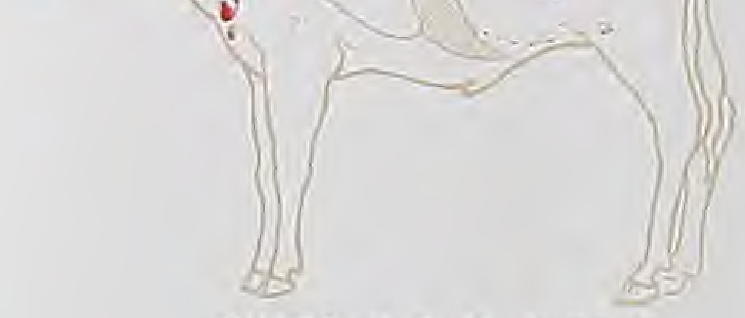
DOSE: 50 MILLIGRAMMES



KILLED: 19. 6. 08. 100 DAYS

620. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 10. 6. 08. 91 DAYS.

CHART 40A.

(*See* Chart 40.)

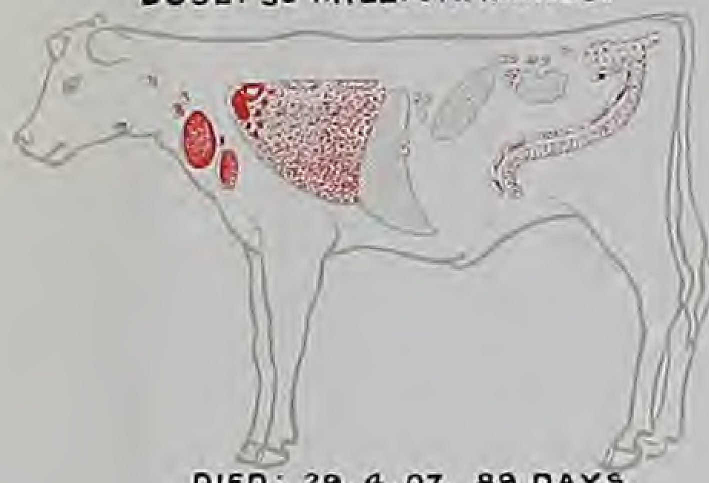
INOCULATION EXPERIMENTS ON CALVES WITH CULTURE OF VIRUS E.IV.

PASSAGE EXPERIMENTS β AND γ

The strain was derived from the original material through a guineapig, and was inoculated on January 30 1907, when it had been 390 days in artificial cultivation.

498. SUBCUT.

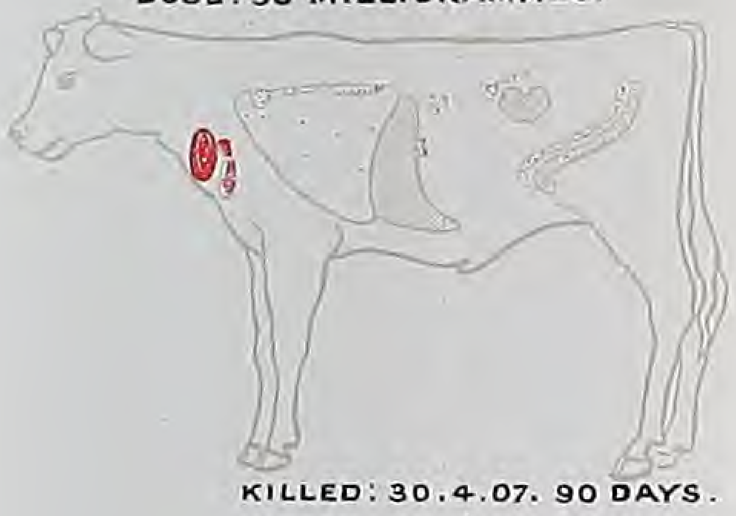
DOSE: 50 MILLIGRAMMES.



DIED: 29.4.07. 89 DAYS.

502. SUBCUT.

DOSE: 50 MILLIGRAMMES.



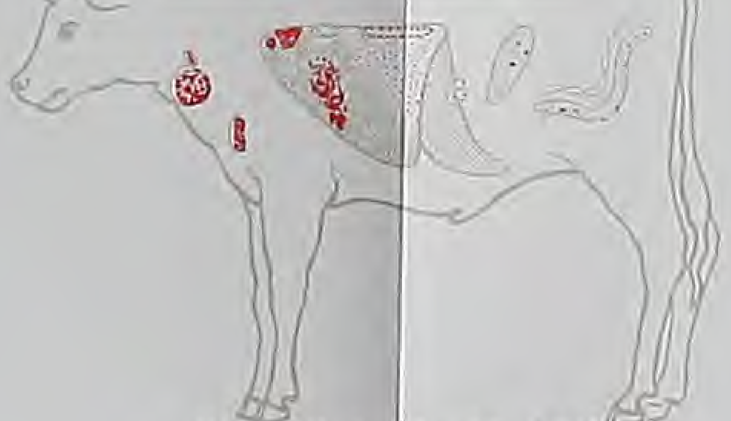
KILLED: 30.4.07. 90 DAYS.

CULTURE.

Derived from the lung of Calf 502; inoculated on May 7, 1908. after 373 days artificial cultivation.

614. SUBCUT.

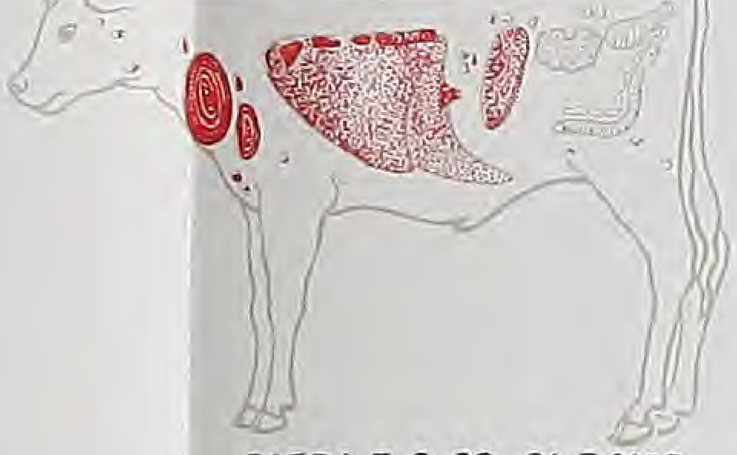
DOSE: 50 MILLIGRAMMES.



DIED: 9.6.08. 33 DAYS.

630. SUBCUT.

DOSE: 50 MILLIGRAMMES.



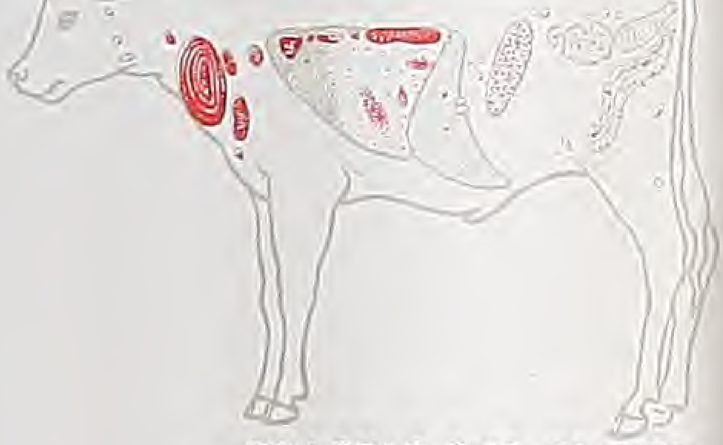
DIED: 7.6.08. 31 DAYS.

CULTURE.

Derived from the suprarenal body of Calf 502; inoculated on April 7, 1908. after 343 days artificial cultivatio

602. SUBCUT.

DOSE: 50 MILLIGRAMMES.



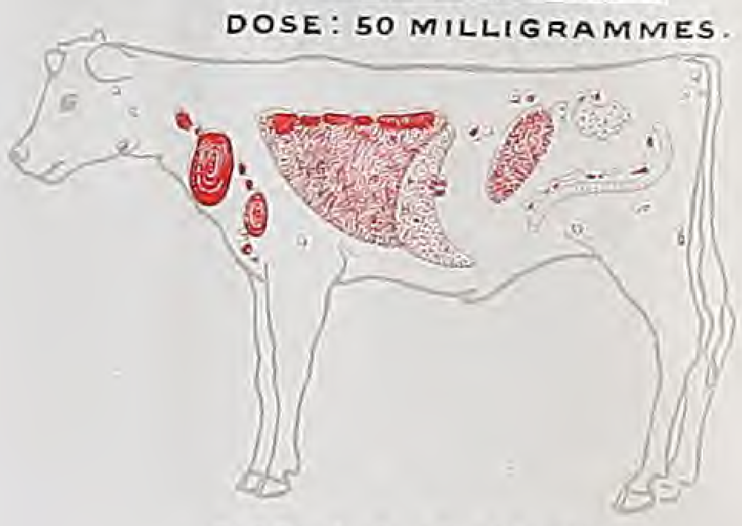
KILLED: 6.7.08. 90 DAYS.

CULTURE.

Derived from the mediastinal gland of Calf 602; inoculated on August 5, 190 after 30 days artificial cultivation.

650. SUBCUT.

DOSE: 50 MILLIGRAMMES.



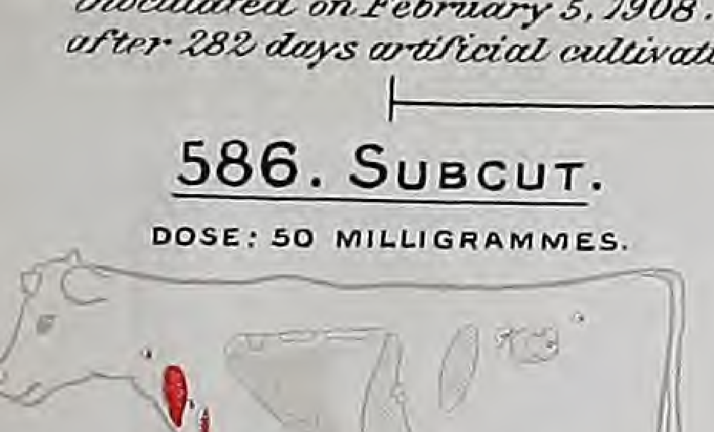
DIED: 13.9.08. 39 DAYS.

CULTURE.

Derived from the lung of Calf 498; inoculated on February 5, 1908. after 282 days artificial cultivation

586. SUBCUT.

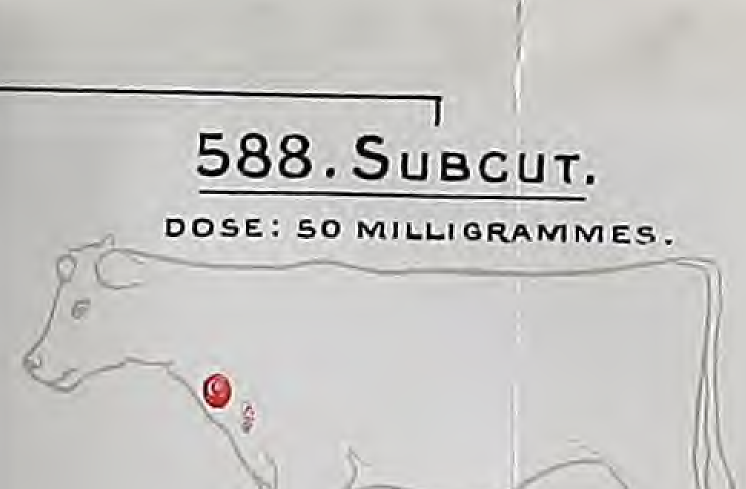
DOSE: 50 MILLIGRAMMES.



DIED (OF BRONCHO-PNEUMONIA) 12.3.08. 36 DAYS.

588. SUBCUT.

DOSE: 50 MILLIGRAMMES.



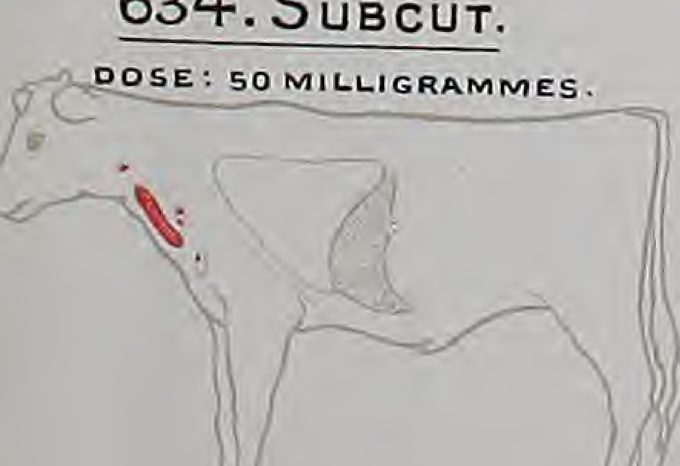
KILLED: 5.5.08. 90 DAYS.

CULTURE.

Derived from the mediastinal gland of Calf 586; inoculated on May 19. 1908. after 68 days artificial cultivation.

634. SUBCUT.

DOSE: 50 MILLIGRAMMES.



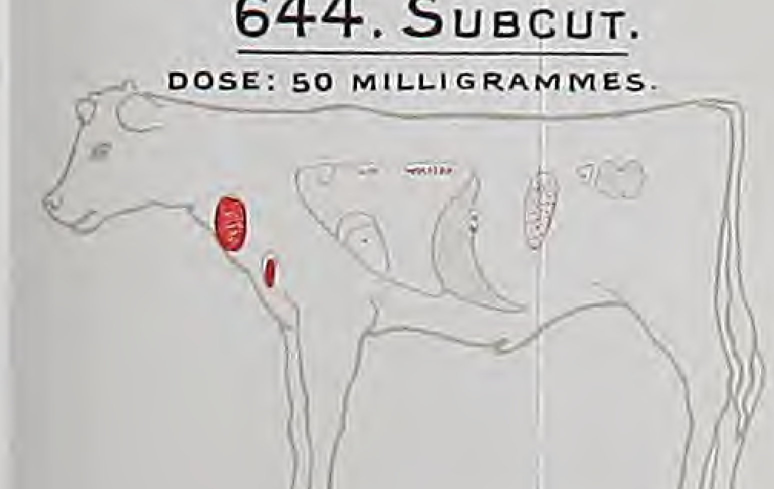
KILLED: 31.8.08. 104 DAYS.

CULTURE.

Derived from the prescapular gland of Calf 588; inoculated on May 29, 1908. after 24 days artificial cultivation.

644. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 1.9.08. 95 DAYS.

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CHARTS 41 and 41A.

Inoculation Experiments on Calves and Baboons with Culture of Virus E. IV.

[Appendix to Final Report, Vol. IV. : "Investigation of Tubercle Bacilli derived from Tuberculosis occurring naturally in the Horse."]

Virus E. IV. was obtained on December 19, 1905, from the spleen of a horse, which showed on post-mortem examination no sign of tuberculosis elsewhere.

Explanation of Charts.—Chart 41 shows diagrammatically (i) the effect on (four) calves (Nos. 408, 410, 616, and 620) and (two) baboons (one fed) of the inoculation of the culture derived from the original material; (ii) the results obtained in one (*a*) of the three passage experiments undertaken to determine whether the virulence of the virus for the calf was stable or capable of being raised by residence in the body of the calf; and (iii) the virulence of the virus for the calf after residence in the body of the baboon (Baboon 74).

Chart 41A shows the effect on other (two) calves (Nos. 498 and 502) of the inoculation of the culture derived from the original material, and the results obtained in two additional passage experiments (β and γ) carried out with this virus.

References.—Final Report: Pages 21 to 24, and 31.

Appendix to Final Report, Vol. IV. : Pages 7, 10, 13, 73 to 91, and 123 to 136.

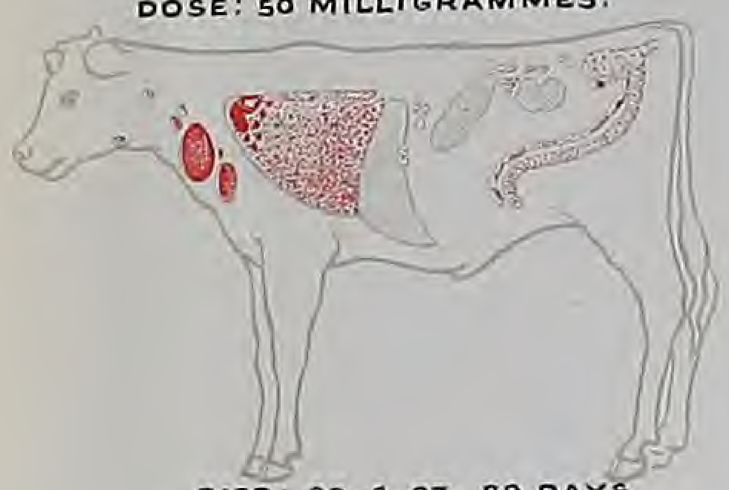
INOCULATION EXPERIMENTS ON CALVES WITH CULTURE OF VIRUS E. IV.

PASSAGE EXPERIMENTS β AND γ

The strain was derived from the original material through a guineapig, and was inoculated on January 30 1907, when it had been 390 days in artificial cultivation.

498. SUBCUT.

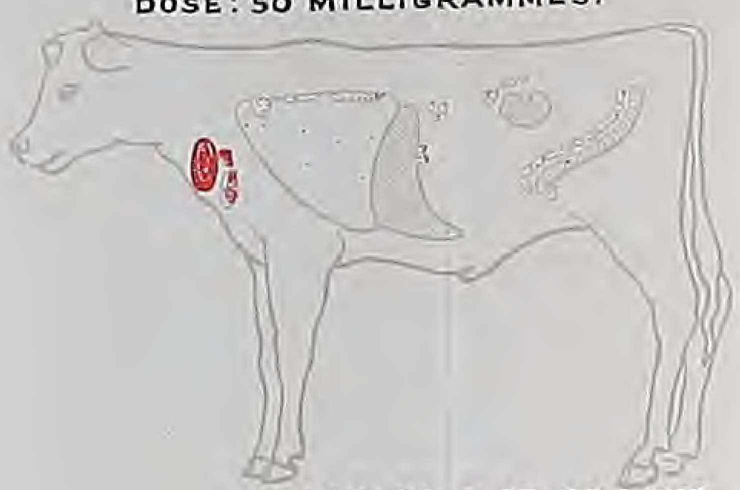
DOSE: 50 MILLIGRAMMES.



DIED: 29.4.07. 89 DAYS.

502. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 30.4.07. 90 DAYS.

CULTURE.

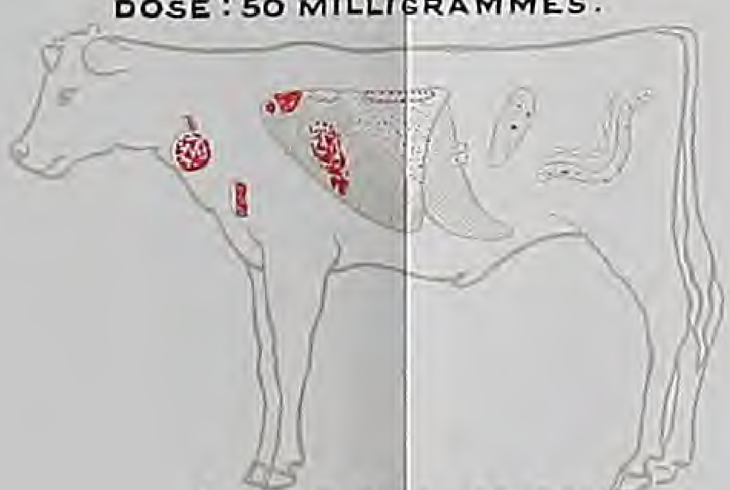
Derived from the lung of Calf 502; inoculated on May 7, 1908. after 373 days artificial cultivation.

CULTURE.

Derived from the suprarenal body of Calf 502; inoculated on April 7, 1908. after 343 days artificial cultivat

614. SUBCUT.

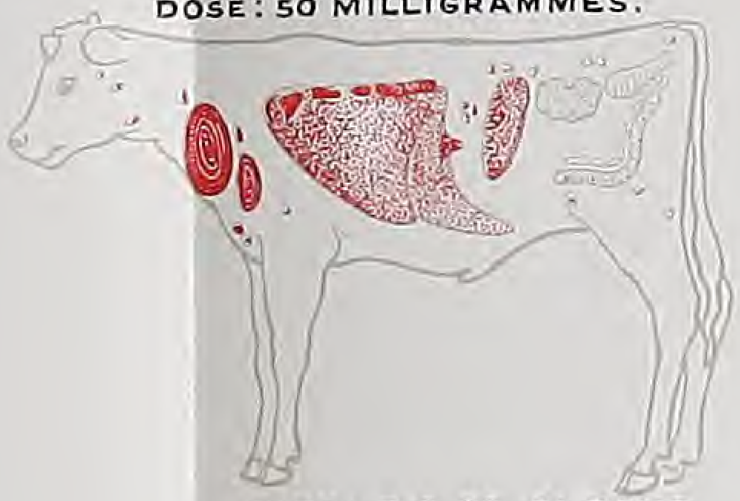
DOSE: 50 MILLIGRAMMES.



DIED: 9.6.08. 33 DAYS.

630. SUBCUT.

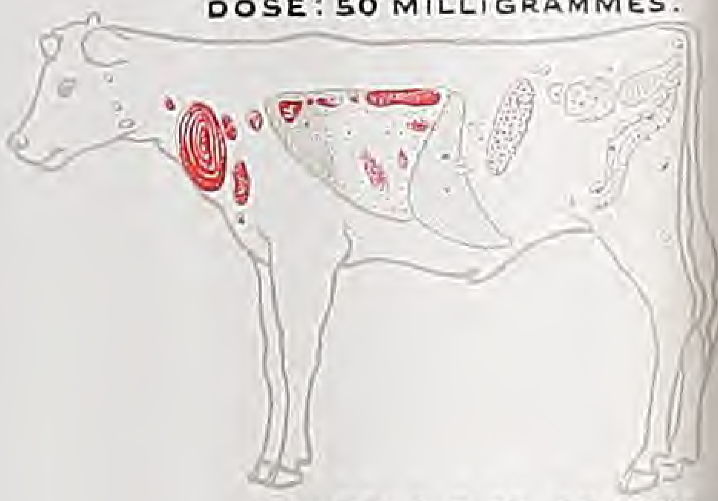
DOSE: 50 MILLIGRAMMES.



DIED: 7.6.08. 31 DAYS.

602. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED: 6.7.08. 90 DAYS.

CULTURE.

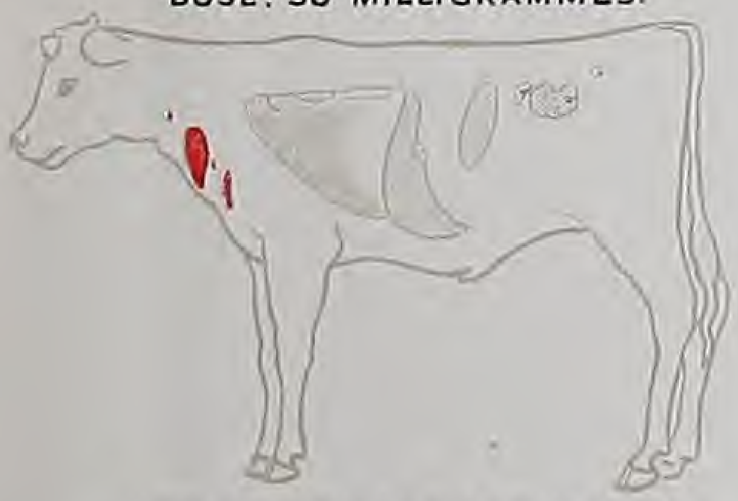
Derived from the lung of Calf 498; inoculated on February 5, 1908. after 282 days artificial cultivation

CULTURE.

Derived from the mediastinal gland Calf 602; inoculated on August 5, 19 after 30 days artificial cultivation.

586. SUBCUT.

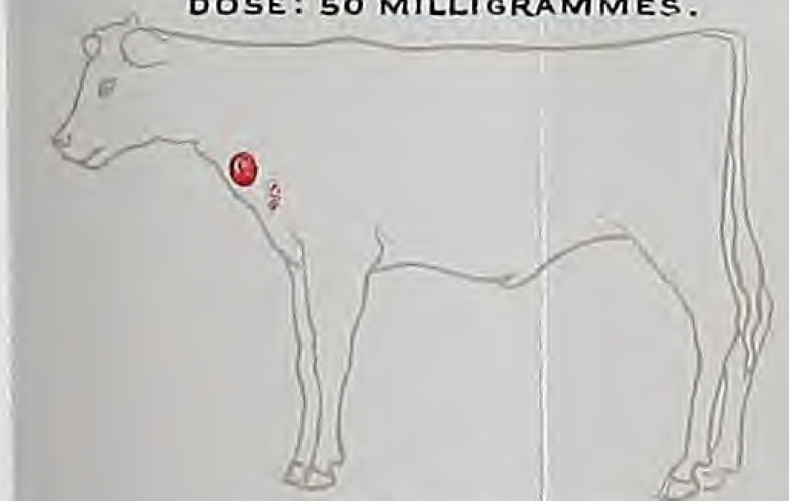
DOSE: 50 MILLIGRAMMES.



DIED (OF BRONCHO-PNEUMONIA) 12.3.08. 36 DAYS.

588. SUBCUT.

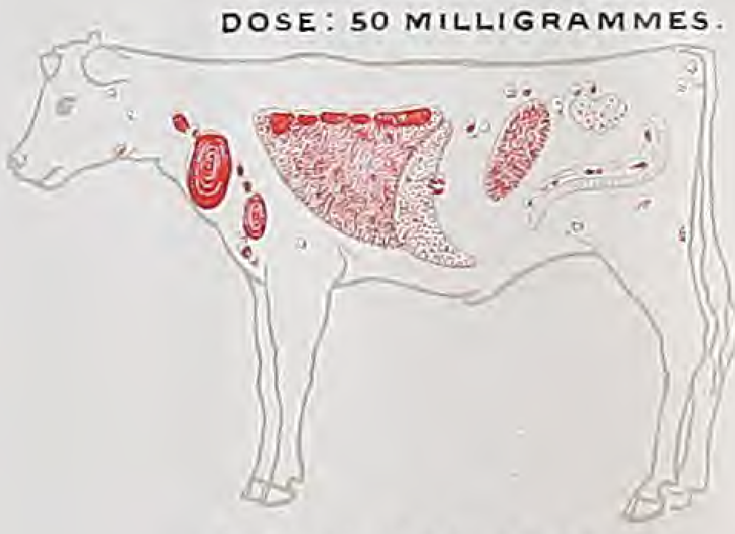
DOSE: 50 MILLIGRAMMES.



KILLED: 5.5.08. 90 DAYS.

650. SUBCUT.

DOSE: 50 MILLIGRAMMES.



DIED: 13.9.08. 39 DAYS.

CULTURE.

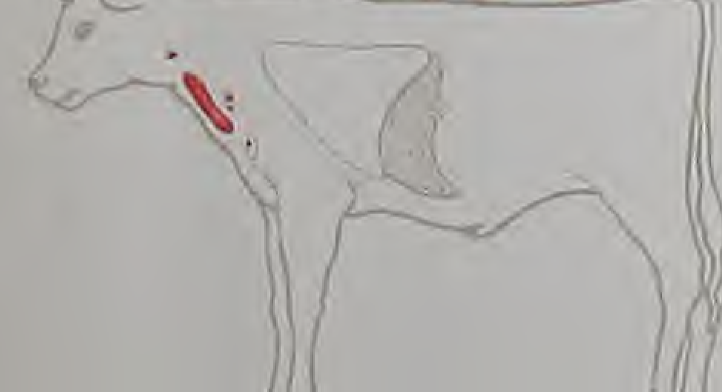
Derived from the mediastinal gland of Calf 586; inoculated on May 19. 1908. after 68 days artificial cultivation.

CULTURE.

Derived from the prescapular gland of Calf 588; inoculated on May 29, 1908. after 24 days artificial cultivation.

634. SUBCUT.

DOSE: 50 MILLIGRAMMES.



644. SUBCUT.

DOSE: 50 MILLIGRAMMES.

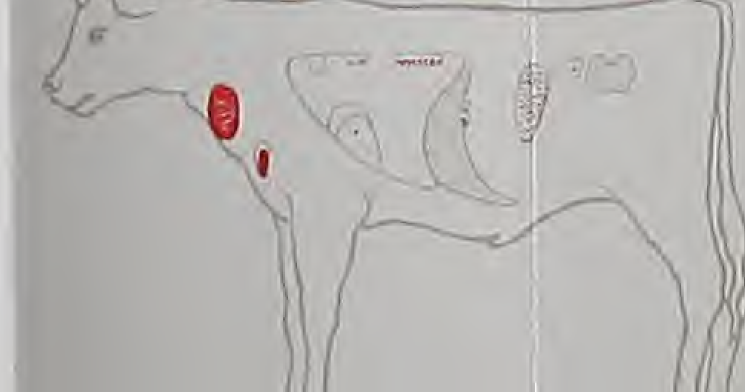


CHART 41A.

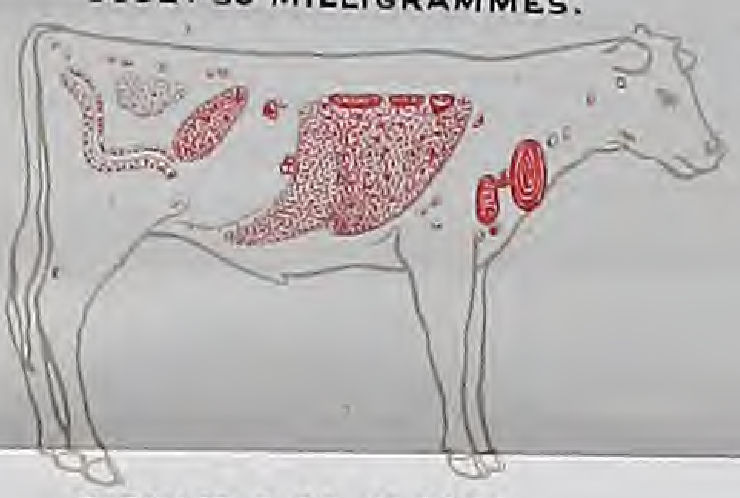
(*See* Chart 41.)

INOCULATION EXPERIMENTS ON CALVES WITH CULTURE OF VIRUS E. I.

The strain was derived from the original material through a guineapig and was inoculated on April 17. 1907. when it had been 482 days in artificial cultivation.

522. SUBCUT.

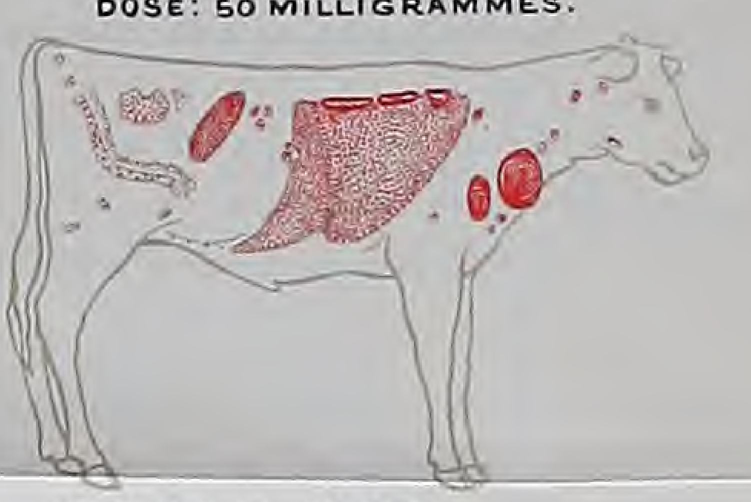
DOSE: 50 MILLIGRAMMES.



DIED: 23. 5. 07. 36 DAYS.

528. SUBCUT.

DOSE: 50 MILLIGRAMMES.



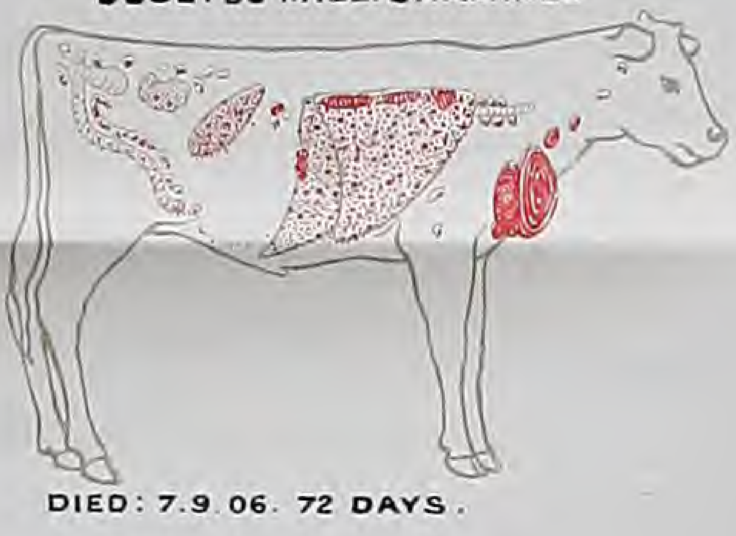
DIED. 17. 5. 07. 30 DAYS.

INOCULATION EXPERIMENTS ON CALVES WITH CULTURE OF VIRUS E. III.

The strain was derived from the original material through a guineapig, and was inoculated on June 27. 1906 when it had been 188 days in artificial cultivation.

440. SUBCUT.

DOSE: 50 MILLIGRAMMES

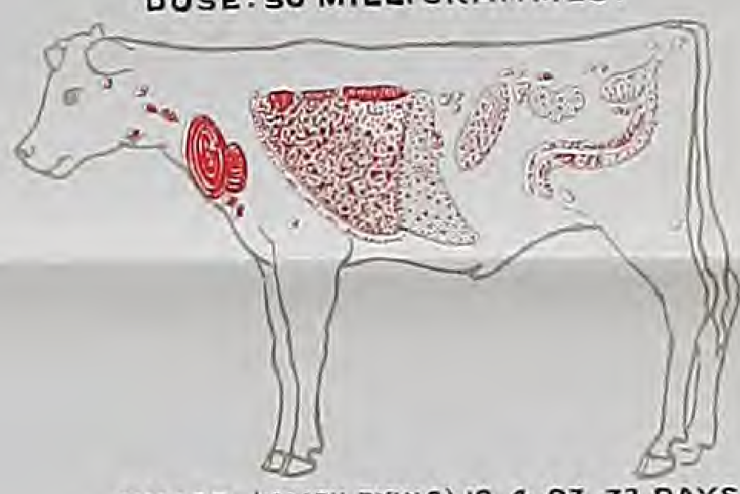


DIED: 7. 9. 06. 72 DAYS.

The strain was derived from the original material through a guineapig and was inoculated on February 4. 1907. when it had been 410 days in artificial cultivation

508. SUBCUT.

DOSE: 50 MILLIGRAMMES.



KILLED (WHEN DYING) 18. 4. 07. 73 DAYS.

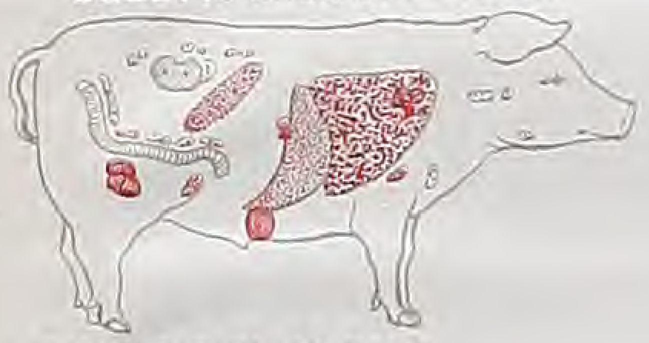
INOCULATION EXPERIMENTS ON PIGS WITH VIRUSES E. I., E. II., E. III., AND E. IV.

VIRUS E. I,

The strain was derived from the original material through a guineapig, and was inoculated on June 20. 1907. when it had been 545 days in artificial cultivation

192. SUBCUT.

DOSE: 10 MILLIGRAMMES.



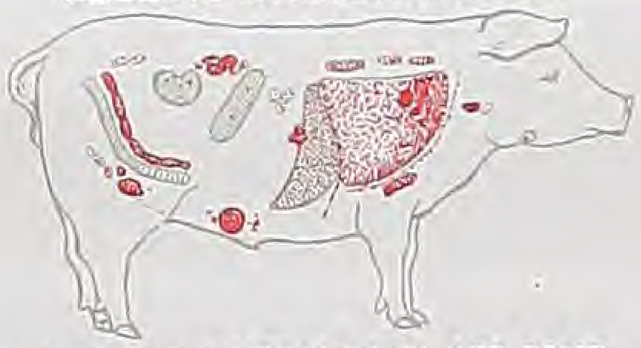
DIED: 7. 8. 07. 48 DAYS

VIRUS E. II.

The strain was derived from the original material through a guineapig, and was inoculated on May 15, 1907. when it had been 510 days in artificial cultivation,

184. SUBCUT.

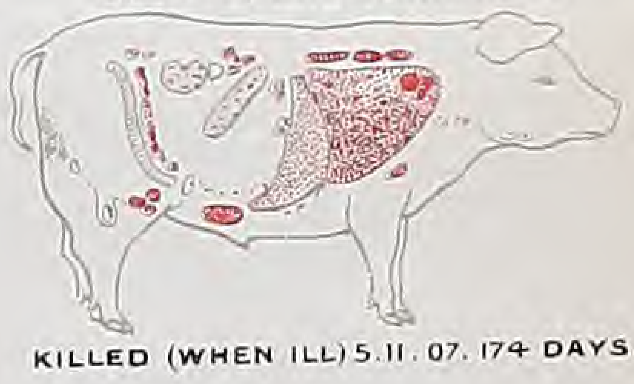
DOSE: 10 MILLIGRAMMES.



KILLED (WHEN WELL) 7. 2. 08. 268 DAYS.

186. SUBCUT.

DOSE: 50 MILLIGRAMMES.



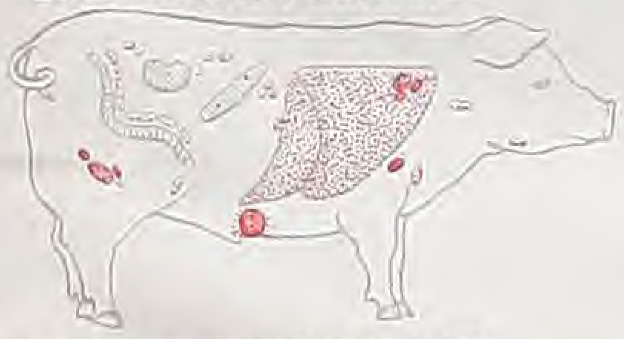
KILLED (WHEN ILL) 5. 11. 07. 174 DAYS

VIRUS E. III.

The strain was derived from the original material through a guineapig, and was inoculated on June 20,1907, when it had been 546 days in artificial cultivation

194. SUBCUT.

DOSE: 10 MILLIGRAMMES



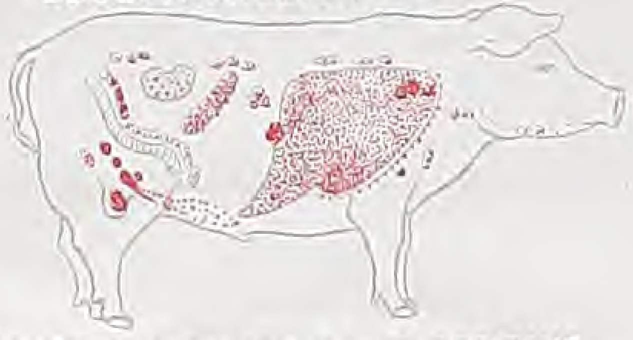
KILLED (WHEN VERY ILL) 22. 8. 07.
63 DAYS

VIRUS E. IV.

The strain was derived from the original material through a guineapig, and was inoculated on May 15. 1907. when it had been 495 days in artificial cultivation

188. SUBCUT.

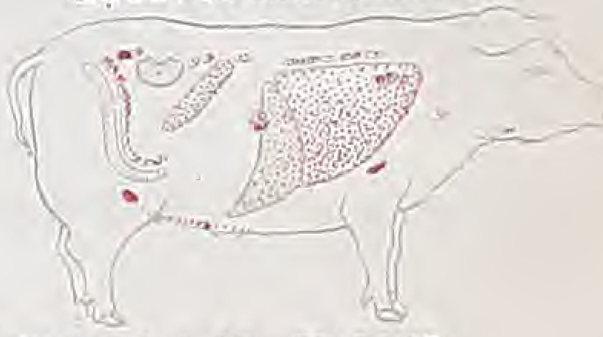
DOSE: 10 MILLIGRAMMES.



KILLED: (WHEN WELL) 7. 2. 08. 268 DAYS

190. SUBCUT.

DOSE: 50 MILLIGRAMMES



KILLED: 6. 11. 07. 175 DAYS.

CHART 42.

(i) "Inoculation Experiments on Calves with Culture of Virus E. I."

Virus E. I. was obtained on December 19, 1905, from the mesenteric glands of a horse which showed extensive tuberculous lesions in the mesenteric glands and spleen, and miliary tuberculosis of the lungs. The virus had the cultural characters of a bovine tubercle bacillus.

References.—Final Report : Pages 21 to 24.

Appendix to Final Report, Vol. IV. : Pages 7, 10, 12, and 39 to 46.

(ii) "Inoculation Experiments on Calves with Culture of Virus E. III."

Virus E. III. was obtained on December 19, 1905, from the enormously enlarged tuberculous mesenteric glands of a horse. The virus had the cultural characters of a bovine tubercle bacillus.

References.—Final Report : Pages 21 to 24.

Appendix to Final Report, Vol. IV. : Pages 7, 10, 12, and 67 to 72.

iii. Inoculation Experiments on Pigs with Viruses E. I., E. II., E. III., and E. IV.

The two viruses E. II. and E. IV. which have, like E. I. and E. III., the cultural characters of the bovine tubercle bacillus, but which have been shown to possess a lower degree of virulence for the calf than the typical bovine viruses E. I. and E. III. (*see* Charts 40 and 41), are here shown to possess also a lower degree of virulence for the pig (compare the length of life of pigs 184 to 190 with that of pigs 192 and 194).

References.—Final Report : Pages 21 to 24 and 31.

Appendix to Final Report, Vol. IV. : Pages 7, 12, 14, 19.

ROYAL COMMISSION ON TUBERCULOSIS (HUMAN AND ANIMAL).

FINAL REPORT OF THE ROYAL COMMISSION

APPOINTED TO INQUIRE INTO THE RELATIONS OF

HUMAN AND ANIMAL TUBERCULOSIS.

PART II. APPENDIX.

VOLUME VII.

GRAPHIC CHARTS

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From 1902 to 1912.

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